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IMMUNOLOGIC STUDIES IN EXPERIMENTAL ENCEPHALITIS *

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A growing tendency has existed among some investigators to attach more and more significance to the herpes virus as a possible cause of epidemic encephalitis. Various workers have reported the isolation of herpes strains from the brain substance in frank cases of epidemic encephalitis. This has been particularly true in parts of the continent and in England. For example, it is generally assumed that the viruses reported by Levaditi, Schnabel and Doerr [1] are true instances of the presence of herpes virus in human encephalitis. Perdrau,[2] apparently, has experienced unusual success in this regard, while others, whose reports have been commented on elsewhere,[1] have described experiments in which viruses were found in human encephalitis, but are not as yet definitely classified. Takaki [3] studied an epidemic of encephalitis in Japan and succeeded in six transmissions of a virus disease to rabbits, with material taken from six fatal cases. Symptomatically and immunologically, the Takaki virus appears to be unlike the viruses of the herpes group. However, the pathologic picture in the Japanese epidemic is similar to that found in the European and American epidemics. Flexner [4] has recently brought together in a most critical analysis recent work on epidemic encephalitis and simple herpes, and restates his position in the matter (Flexner and Amoss),[5] namely, that

* From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

* This work as well as our previous contributions on this subject was supported by the W. D. Wood Fund.

1. McKinley, E. B., and Holden, M.: J. Infect. Dis. **39**:441-450, 1926.
2. Perdrau, J. R.: Brit. J. Exper. Path. **6**:41, 1925.
3. Takaki, I.: Ztschr. Immunitätsforsch. u. exper. Therap. **47**:441, 1926; Japan M. World **5**:147, 1925.
4. Flexner, S.: J. General Physiol. **8**:713-735, 1925.
5. Flexner, S., and Amoss, H. L.: J. Exper. Med. **41**:233 and 357, 1925.

the true virus of encephalitis remains undiscovered and that the presence of herpes virus in human encephalitis can be explained on the basis of its accidental presence only. Flexner is forced to this conclusion in view of the fact that examination of material from a large series of cases of encephalitis failed to reveal a herpes virus, while success was achieved in only one instance with material taken from a case of neurosyphilis.

Aside from the objections interposed by Flexner, a series of histologic facts fortifies his position. In general, these are well known and have been repeatedly described, though the presence of inclusion bodies in the experimental disease in rabbits with herpes virus and their definite absence in human epidemic encephalitis has not been emphasized sufficiently as a point of dissimilarity. Likewise, the confusion brought about by the discovery of spontaneous encephalitis in experimental animals has tended to minimize much of the experimental data presented.

Attention has already been called to the immunologic aspect of the problem.[1] Zinsser and Tang,[6] we and others have prepared immune serums in rabbits with herpes strains that were capable of neutralizing the herpes virus in vitro. But Zinsser and Tang also find that the serum from encephalitis patients does not have a neutralizing effect on the herpes virus. As a result of their investigations, these authors conclude that epidemic encephalitis is not related to herpes. However, they point out that irregularities which have been reported with immune serums and their virucidal action on the herpes virus might be explained by the fact that some of the virus is extracellular, in which case it is destroyed, and that some of the virus is intracellular, in which case it would be protected from the action of the serum. The same explanation, of course, might hold for the negative results obtained with serum from encephalitis when tested in vitro.

To our minds, there is another possibility of the mechanism of infection in epidemic encephalitis which is not wholly incompatible with the theory of epidemic encephalitis of herpetic origin. The fact that only in rare instances has herpes virus, or any virus for that matter, been recovered from human cases either before death or after death suggests the possibility that the causative agent is not to be found, except in rare instances, in the central nervous system, and further that it is unnecessary to discover its presence there. It is well known that a form of "brain inflammation" is produced in botulism, which is apparently due to a neurotoxin. We have at hand in the work of Goodpasture and Teague[7] some evidence that the herpes virus selects the axis cylinder of the nerve trunk as its route for reaching the central nervous system from a peripheral muscle. It is possible, though with the

6. Zinsser, H., and Tang, F. F.: J. Exper. Med. **44**:21, 1926.
7. Goodpasture, E. W., and Teague, O.: J. M. Research **44**:121, 1923.

present knowledge of filtrable virus toxins it is purely hypothetic, that the herpes virus may produce encephalitis by virtue of a neurotoxin acting from a focus. And further, in those cases of encephalitis that follow in the wake of some other infection, such as influenza, for example, the virus might penetrate the central nervous system by virtue of having a prepared soil. Such cases could conceivably account for the presence of herpes in the brain substances, especially in the acute stages of the disease. In order further to defend such a hypothesis, it would be necessary to assume that the herpes virus is fairly ubiquitous and the possibility of latency of the herpes virus in the peripheral nerve trunks and ganglions or elsewhere in the body. Such considerations would lend themselves readily to a simple explanation of recurrent herpes. If the virus is capable of passing up the nerve trunk, it is at least possible that it may travel the other way as well. Emotional stress is frequently spoken of as related to recurrences of herpes, and the herpes virus used in the experiments, which will be described, came from such a case.

To support such a hypothesis as we have advanced, then, two factors must be present: first, the herpes virus, and second, a prepared soil. The prepared soil is limited only to the causes of disease, whether major or minor, in their intensity and effects. We do not intend to defend such a hypothesis, but simply to advance it, and suggest a method through immunologic studies with the herpes virus by which, in part, such a theory might be tested clinically.

A previous communication described a method of immunizing rabbits with herpes virus that produced a serum highly virucidal in the test tube.[1] Rabbits immunized against the LeFèvre herpes virus and the Levaditi virus were found to be immune to the homologous virus, and cross immunity was demonstrated. The serum from an animal immune to the Levaditi virus was found to be virucidal for both the herpes virus and the Levaditi virus. These experiments have been extended to determine whether the serum of a herpes immune animal is virucidal for the herpes virus, and, further, whether such a serum, which has proved virucidal in the test tube, will produce a passive immunity against subdural injections of the herpes virus in normal rabbits. An attempt has also been made to immunize sheep against the herpes virus, and similar tests for virucidal effect and passive immunity have been studied. Finally, we have studied the immunologic relationship between vaccinia and herpetic encephalitis.

ACTIVE IMMUNITY WITH HERPES VIRUS IN RABBITS

Ten rabbits were immunized against the herpes virus, receiving first six subcutaneous injections of a serum-virus mixture, followed by four injections of fresh virus in saline. The serum-virus mixture

injections were as follows: two injections of 1 cc. each of a dilution of 1:1000; two injections of 1 cc. each of a dilution of 1:100; one injection of 1 cc. of a 1:10 dilution, and, finally, one treatment with 1 cc. of concentrated virus in serum. The following four injections consisted of 1 cc. of fresh virus emulsified in saline. The intervals between injections were three and four days. It should be mentioned that in each instance the fresh virus was tested subdurally in rabbits and produced a typical encephalitis.

It will be noted in table 1 that this series of rabbits was subsequently tested subdurally for immunity, and that all survived, while the controls died within four days with the typical disease. This experiment serves to confirm our previous work that an active immunity can be produced in rabbits by the method employed.

TABLE 1.—*Active Immunization with Herpes Virus*

	Number and Amount of Injections		Period of Immunization		Test Subdural Fourteen Days After Last Injection	
Rabbits	Serum* Virus Mixture	Fresh Virus in Saline Solution	Total Time	Intervals Between Injections		Results
1-6	6 of 1 cc.	4 of 1 cc.	37 days	3-4 days	0.2 cc. H5318	Survived
7-10	4 of 1 cc.	4 of 1 cc.	26 days	3-4 days	0.2 cc. H4953	Survived
11	.....	.....	.....	.....	0.2 cc. H5318	Died with encephalitis after four days
12	.....	.....	.....	.....	0.2 cc. H4953	Died with encephalitis after four days

* Dilutions in order of 1:1000, 1:100, 1:10, concentrated.

VIRUCIDAL EFFECT OF ANTIHERPES SERUM IN VITRO

In order to obtain a sample of immune serum that was prepared in rabbits and that would be of sufficient quantity for an attempt to produce passive immunity in a group of normal rabbits, it was thought best to pool the serum from two of the active immune animals. Accordingly, after the actively immune animals had survived several weeks, the two rabbits were bled to death, and the serum was mixed for preliminary tests of virucidal effect in vitro. The control animals in this series, which received fresh herpes emulsion in normal serum, died within four days. Two rabbits that received herpes virus-normal rabbit serum mixture after twenty-four hours in the incubator died in six and seven days, respectively. This is a delay over the usual time of death with this virus, but it illustrates that at this temperature for twenty-four hours the virus is not destroyed when emulsified in normal serum. Four of the remaining animals, which received a mixture of the herpes emulsion and the pooled immune serum after twenty-four hours contact in the incubator, survived the subdural inoculations. One rabbit was apparently injured during the inoculation and died in two days, while another rabbit died in eight days.

PASSIVE IMMUNITY WITH ANTIHERPES SERUM IN RABBITS

It was assumed from these experiments that the pooled immune serum was virucidal in vitro for the herpes virus. On this basis, then, it was thought advisable to attempt the production of passive immunity in normal animals by intravenous treatment with immune serum. Passive immunity was found to be produced easily in this manner. In one experiment five rabbits were protected against subdural inoculation of fresh herpes emulsion (0.2 cc.) after having previously received three intravenous injections of 5 cc. each of the pooled immune serum, at seven day intervals. The animals were tested for immunity three weeks after the last intravenous injection of the immune serum. All of them survived, and the controls died in four and six days, respectively. These animals were retested by subdural injections of fresh herpes emulsion thirty-two days later, and all succumbed within from three to six days after inoculation. This seems to indicate that the passive immunity so readily established is of a short duration.

TABLE 2.—*Passive Immunity Produced in Rabbits with Pooled Immuned Serum* *

Rabbits	Intravenous Injection of Immune Serum	Subdural Test 3 Weeks After Last Injection	Result
1-5	3 injections of 5 cc. each	0.2 cc. virus H5348	Survived 32 days
6, control	.....	0.2 cc. virus H5348	Died in 4 days
7, control	.....	0.2 cc. virus H5348	Died in 6 days

* The animals again received subdural injections thirty-two days later and all died with typical encephalitis within from three to six days.

RELATION OF VACCINE VIRUS IMMUNITY TO HERPES ENCEPHALITIS

Turnbull and McIntosh,[8] Heymann [9] and Aldershoff [10] have brought up the question of the relation of encephalitis to vaccination against smallpox. Heymann quotes Jenner's observation on inhibition of the development of the smallpox vaccine pustule in patients with herpes. Turnbull and McIntosh have reviewed seven cases of encephalomyelitis which were definitely connected with vaccination. The only virus demonstrated experimentally in the brain and cord was a vaccinal virus. The postvaccinal encephalitis develops from nine to fifteen days after the vaccination. Levaditi regards this form of encephalitis as the flaring up of a latent virus of epidemic encephalitis already present in the central nervous system. Perhaps the theory of the prepared soil is applicable here.

With these observations in mind, we have vaccinated with typical result a group of nine rabbits and tested them later by subdural inocula-

8. Turnbull, H. M., and McIntosh, I.: Brit. J. Exper. Path. **7**:181, 1926.
9. Heymann, B.: Deutsche med. Wchnschr. **52**:442, 1926.
10. Aldershoff, H.: Nederl. Tijdschr. v. Geneesk. **2**:267, 1826.

tions with fresh herpes virus emulsions (0.2 cc.). As was to be expected, immunity was not established against the herpes virus by virtue of vaccine virus immunity. All of the animals, including the two controls, died within from three to six days. Busson [11] only recently reported that guinea-pigs inoculated with cowpox virus were immune to infection with the street virus of rabies. This is decidedly not true for herpes virus.

IMMUNIZATION OF SHEEP WITH HERPES VIRUS

It is well known that many different species of animals are subject to special forms of encephalitis. Horses, dogs, rabbits and, more recently, sheep are said to have forms of encephalitis which may even appear in epidemics. We attempted by various methods to immunize sheep to herpes virus, but with only partial success. By daily subcutaneous injections in sheep of serum-herpes virus mixtures, followed later by fresh herpes emulsion in amounts as large as 5 cc., it was possible to obtain some evidence of immunity. The interval method of immunization completely failed to give any such evidence. Not until the sheep had received daily injections of fresh virus for twenty-one days was any evidence obtained. The serum from such animals, taken two weeks after the last injection, was found to possess some virucidal effect for the herpes virus in vitro, although the protocol is not by any means satisfactory and one hesitates to draw conclusions from it. Such antiserums for the herpes virus prepared in sheep do not confer a passive immunity in rabbits, as do the antiserums prepared in rabbits. If antibodies were produced at all they were present in extremely small amounts.

COMMENT

While the evidence brought forward regarding the relation of herpes virus to epidemic encephalitis is totally insufficient at present to enable us to assume that there is any connection between the two, the conservative attitude in the present state of knowledge apparently is to treat the question with an open mind. It seems strongly indicated that epidemic encephalitis is caused by a filtrable virus. Whether the lesions characteristic of this disease are caused by the presence of the herpes virus or of any other hypothetic virus in the central nervous system, or by similar viruses concentrated elsewhere and acting as a focus, is, under present conditions, purely a matter of conjecture. The fact remains that herpes viruses have been isolated from the brain substance in frank cases of encephalitis. If these results are to be regarded as purely accidental, one must assume that through some mechanism their passage into the central nervous system has been made possible.

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11. Busson, B.: Wien. klin. Wchnschr. **39**:1183, 1926.

LeFèvre and Millet [12] have shown that intravenous sublethal doses of herpes virus in rabbits become lethal when the inoculation is preceded by the injection of certain dyes and drugs which, these authors believe, alter the permeability of the meninges. It is possible, of course, that the lesions in epidemic encephalitis would so alter the permeability of the meninges as to allow entrance of the herpes virus if it were latent elsewhere in the body. This would explain the accidental finding of herpes, then, in some cases of epidemic encephalitis.

On the other hand, if one considers the possibility that the herpes virus is itself related to epidemic encephalitis, how can one account for its rare occurrence in the central nervous system? To revert to the idea advanced in the beginning of this paper, it would appear that the presence of the herpes virus in the central nervous system is not an absolute essential; that the effect is produced not by its presence, but through some other activity. If this is true, one can well understand how, given the prepared soil, in certain cases the virus could pass through the normal barriers and be found in the central nervous system of an isolated case from time to time.

Tests for etiology based on therapeutics have long been open to criticism. However, here is a disease in which no definite etiologic agent has presented itself. If there is the remotest possibility that the virus of herpes and that of encephalitis are identical, a serum that has been shown to have antagonistic properties and that confers passive immunity against herpes might be tried in the treatment of cases of encephalitis.

CONCLUSIONS

Confirmation of our previously published method of producing active immunity in rabbits against the herpes virus is presented. Serums from such actively immunized rabbits were found to be virucidal in vitro for the herpes virus. Passive immunity was produced in rabbits with serum from actively immunized animals. Evidence of active herpes immunity produced in sheep is cited, though serums from such sheep do not produce passive immunity in rabbits. Data are presented to show that vaccinia and experimental herpes encephalitis are not related.

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12. LeFèvre, M., and Millet, M.: Compt. rend. Soc. de biol. **94**:782, 1926.

CARCINOMA OF THE BREAST COMBINED WITH A GIANT CELL SARCOMA

REPORT OF A CASE *

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So-called multiple or combined malignant tumors occurring in the same organ are rare, and the pathogenesis of such lesions is still disputed. Recent experimental investigations in irritation neoplasms, wherein both epithelial and connective tissue malignancy were observed, have added many interesting observations to the literature.

The case reported here is interesting not only because of the infrequency of the condition, but likewise from the standpoint of its histogenesis.

REPORT OF CASE

Mrs. F., a white woman, aged 43, entered St. Margaret's Hospital on the service of Dr. C. C. Nesselrode, complaining of a painful mass in the right breast which was first noticed eleven months previously as a small lump, about one-half inch (1.27 cm.) in diameter, in the region of the nipple. It was tender at times but became painful only a month prior to entry into the hospital. It gradually enlarged to the size of "an orange." When the mass first became tender she noticed some sensitive "lumps" in the axilla. There was not any loss of weight. There had been a serous and sometimes bloody discharge from the nipple during the past seven months. The patient had pellagra eight years before, when she lived in the south. She also had a severe mastitis in the left breast several years previously. Her mother died of what was thought to be carcinoma of the stomach.

Physical Examination.—The skin was sallow. The mouth showed pyorrhea alveolaris. The right breast was distended by a nodular tumor mass and the overlying skin was adherent and pigmented, and showed some areas of bluish discoloration. The axillary lymph nodes on the right were easily palpable.

At operation a radical amputation of the breast was done, removing the sternal portion of the pectoralis major and minor muscles and the axillary glands. Recovery was uneventful and the patient left the hospital sixteen days after admission.

Three months after dismissal from the hospital she returned with metastatic nodules in the operative scar. Evidence of other involvement was not present. She had not lost weight and was in apparent good health.

Gross Appearances.—The specimen was a right breast measuring 10 by 8 by 6 cm. It was covered by an elliptical piece of skin with the nipple almost centrally located. The skin about the nipple and for a distance of about 4 cm. lateral to it was brown and discolored. Immediately beneath the skin, in the subcutaneous tissue, there was a soft, boggy, bloody mass composed of spongy, hemorrhagic,

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necrotic tissue, which extended from the corium to a distance of about 3 cm. into the breast. The tumor was poorly defined, had replaced almost the entire breast tissue and involved the underlying muscle. On cross section, a nonencapsulated, pale, grayish white, cellular growth was seen invading in all directions, sometimes presenting poorly defined nodules. The growth was soft and friable, particularly near the base of the necrotic zone, while other deeper areas were hard and elastic in character. Areas of hemorrhage and degeneration of the deeper tumor tissue were seen. Here and there islands of fat were encountered. Little normal appearing breast tissue was found. Some red hemorrhagic areas of tumor invasion were seen which had a base of muscle and showed a type of tissue that was red and friable, looking somewhat like granulation tissue. Other areas invading the breast were rather hard and almost scirrhous in character.

Some attached glands were enlarged but on section appeared cellular, pale and edematous. Isolated cellular masses could not be recognized with the naked eye, although they were much more indurated in consistency than normal.

Histologic Pathology.—The picture was variable. The growth was wild and atypical with an irregular glandular type of epithelial structure showing a striking lack of differentiation of the cells invading the surrounding tissue. Evidence of encapsulation was not present. Mitotic figures, bizarre nuclear forms and hypochromatic and hyperchromatic nuclei were abundant. Some cells showed a feeble attempt at gland formation, but for the most part they were undifferentiated, although occasional atypical tumor acini filled with pink granular débris were seen. The variation in cell type and occurrence of nucleated masses of cytoplasm was so marked in some areas that the structure closely resembled that of a rather cellular giant cell sarcoma. Considerable hemorrhage and degeneration had taken place in some fields. The sarcomatous appearing structure was composed of large multinucleated giant cells, some of them containing as many as a hundred nuclei. These giant cells were embedded in a mass of spindle, polygonal and irregularly shaped cells, the former predominating considerably. Some of these cells resembled undifferentiated epithelial cells. They varied considerably in size, shape and staining; some contained abundant faintly pink staining cytoplasm and mitoses were encountered frequently. Some of them were almost giant in size, containing only one nucleus. Numerous small mononuclear leukocytes were seen infiltrating this stroma. In some fields where the mixture of the two pictures was present, large cells of a neoplastic giant cell character were seen; these contained large pale vesicular nuclei which were sometimes piled up or were abutting directly on each other and were much larger and more variable in size than those of the other areas which resembled so closely the multinucleated cells in giant cell sarcoma. In places there seemed to be a transition from the epithelial to the giant cell type of growth, and in many areas of the latter type the cellular stroma closely resembled the cells in the carcinomatous appearing areas. The skin was not infiltrated directly by the tumor cells.

The stroma of the tumor varied considerably, being abundant in some areas and scanty in others, while some sections showed it to be somewhat edematous. In one area where the structure was distinctly of a branching epithelial character, the cells were lying in a loose, stringy stroma which stained a pale blue and appeared to have undergone mucoid degeneration. The dense stroma surrounding the cellular areas showed some fields containing atrophied gland units and considerable infiltration with small round cells, with here and there a few small atrophied nests of atypical epithelial cells.

The lymph nodes showed some fibrosis in places. One gland in particular was partially replaced by tumor cells. In this gland, giant cells, atypical epithelial cells and other forms corresponding to those seen in the mother tumor were encountered.

The recurrent nodules in the scar showed the same picture as the original tumor of the breast, carcinomatous and giant cell areas being seen.

COMMENT

In a review of the literature, twenty-four cases of multiple tumors of the breast were found. In some of these there is considerable confusion in regard to the proper classification, and it seems that a few of them might possibly be grouped among the teratoid mixed tumors. I mention in particular the cases reported by Kaufmann,[1] Wehner,[2] Orth[3] and Dorsch.[4] Unfortunately, in a few of the other cases the histologic reports are either absent or incomplete; hence, it is difficult to classify them accurately.

Many interesting explanations have been advanced to account for these remarkable tumors. Kerbirion[5] in 1879 reported the first case, which was a well encapsulated tumor, histologically both sarcomatous and carcinomatous. He thought that this neoplasm was originally benign and became sarcomatous and later carcinomatous. Dorsch, in a monograph on the subject, considered the tumor he was reporting primarily carcinomatous, with sarcoma arising from stimulation of the subepithelial connective tissue, by action of the malignant epithelial cells. Schlagenhaufer,[6] Orth and Hedren[7] believed that their cases represented conditions wherein there was a simultaneous development of both carcinoma and sarcoma. Schwartz,[8] on finding a suspicious area in a uterine myoma, suggested that the sarcoma may have been metastatic. Tokano[9]

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1. Kaufmann, E.: Lehrbuch d. Speziellen Pathologische Anatomie, ed. 5, Berlin, Georg Reimer, 1909, p. 1,053.

2. Wehner, E.: Ein Beiträg. zur Frage der Karzinosarkome unter Mitteilung eines Mammatumors, Frankfürt. ztschr. f. Path. **16**:167, 1914.

3. Orth: Charité-Ann. **34**:21, 1910 (cited by Deaver and McFarland); The Breast: Its Anomalies, Its Diseases and Their Treatment, Philadelphia, P. Blakiston's Son & Co., 1917, pp. 409-413.

4. Dorsch, Moritz: Ueber Carcinom and Sarcom derselben Mamma, Inaug. diss., Würzburg, 1896.

5. Kerbirion and Danel: Sarco-épithéliome kystique du sein, J. d. sc. méd. de Lille **1**:175, 1879.

6. Schlagenhaufer, A.: Carcinom und Riesenzellsarkom derselben Mamma, Centralbl. f. allg. Pathol. u. path. Anat. **17**:385-388, 1906.

7. Hedren, G.: Sarkocarcinom der Mamma, Centralbl. f. allg. Pathol. u. path. Anat. **26**:265-269 (May 31) 1915.

8. Schwarz, E.: Carcinoma and Sarcoma Mammae, Am. J. Obst. & Gynec. **68**:752, 1913.

9. Tokano, N.: Ueber das Carcinoma Sarcomatodes der Mamma, Arch. f. klin. Chir. **103**:155-176, 1914.

concluded that he was dealing with sarcomatous transformation of the carcinomatous stroma.

Since the advent of experimental transplantation and irritation tumor production, many startling observations have been made. Ehrlich and Apolant [10] in 1906, and later Russell,[11] by the retransplantation of adenocarcinomas in mice were able to observe these tumors transform gradually into typical sarcomas. An observation more difficult to explain was that of Kidner,[12] who saw a carcinoma recur in the operative field after the removal of a sarcoma. Fibiger, Fibiger and Bang, Biersch and Moeller, Murray and Woglom, Truffi, Deelman and others [13] have described carcinosarcomas in mice following the painting of tar. In Fibiger's tumor the sarcoma outgrew the carcinoma and after thirty-one generations of transplantation remained sarcomatous. Biersch and Moeller separated the two elements of their tumor and were able to cultivate each. A large amount of literature has been published covering these observations and many interesting hypotheses have been formulated to explain the phenomena.

It is likewise of some interest to find that in the experimental tumors, although the majority of cases have shown only sarcomatous metastasis, both carcinoma and sarcomas combined, as well as carcinoma alone, have been observed. In the cases reported in the female breast Coenen,[14] Orth, Schlagenhaufer and Schwartz found both sarcomatous and carcinomatous metastasis. In other cases pure spindle cell sarcoma has been reported; in still others, scirrhous carcinoma alone was found.

Intimate mixture of the two elements, sharply circumscribed tumors without any admixture and others in which the tumors were apparently invading each other have been reported. The prognosis in those cases that have been followed is bad.

Although in the present state of knowledge it is impossible to make any definite conclusions from the cases reported and the experimental tumor phenomena observed, it seems that one of three explanations will cover most of the reported cases. First, the tumors are true carcinoma sarcomatoides in which carcinomatous parenchyma and sarcomatous stroma are present; second, they represent two distinct tumors, or

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10. Ehrlich, P., and Apolant, H.: Zür Kenntnis der Sarkomentwicklung bei Carcinom-transplantation, Centralbl. f. allg. pathol. u. path. Anat. **17**:513, 1906.

11. Russell, B. R. G.: Sarcoma Development Occurring During the Propagation of a Hemorrhagic Adenocarcinoma of the Mamma of the Mouse, J. Path. & Bact. **14**:344-378, 1909-1910.

12. Kidner, F. C.: Sarcoma and Carcinoma in the Same Patient, Boston M. & S. J. **157**:836, 1908.

13. Cited by Woglom, W. H.: Experimental Tar Cancer, Arch. Path. **2**:709-752 (Nov.) 1926.

14. Coenen, H.: Ueber Mutationsgeschwülste und ihre Stellung im onkologischen System, Beitr. z. klin. Chir. **68**:605, 1910.

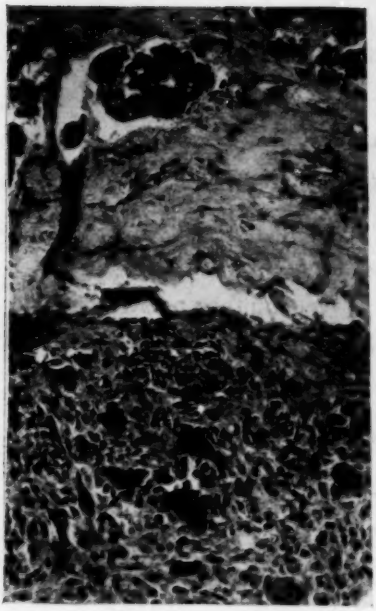


Figure 1

Figure 2

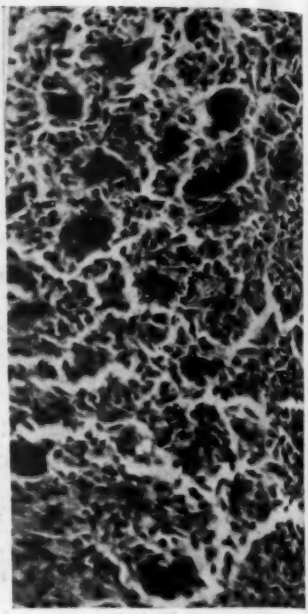


Figure 3

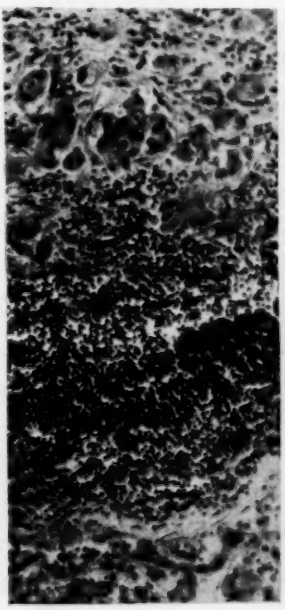
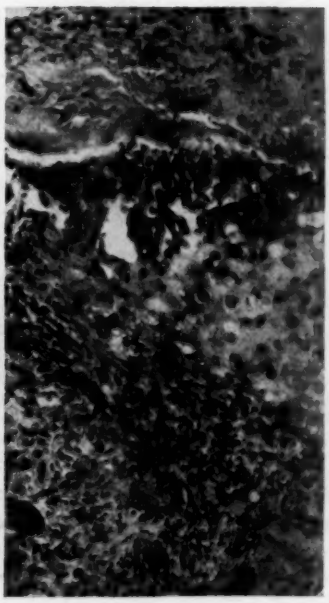


Figure 4



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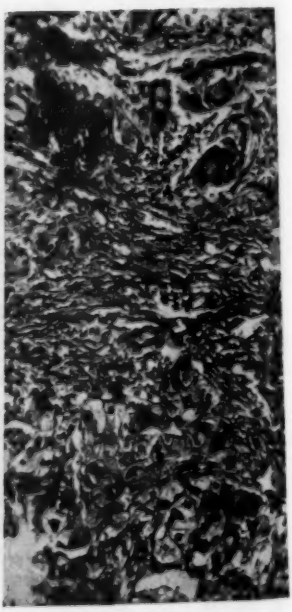

Figure 6

Fig. 1.—Giant cell type of growth at the bottom and solid epithelial nests at the top. × 135.

Fig. 2.—Atypical infiltrating cells which resemble more nearly epithelial structures. × 135.

Fig. 3.—Giant cell type of growth throughout. × 135.

Fig. 4.—A giant epithelial type of metastasis in an axillary lymph gland. × 135.

Fig. 5.—A lymph gland in which both types of growth are present side by side. × 135.

Fig. 6.—Metastatic nodule in the postoperative scar; both giant cells and infiltrating epithelial masses are seen. × 115.

third, a metaplasia has occurred with a gradual transition from carcinoma into a sarcoma-like structure. The case reported here is best explained by the last theory.

CONCLUSION

A case of carcinoma of the breast showing what was thought to be a sarcomatoid metaplasia is reported. The histologic picture was that of carcinoma and giant cell sarcoma. Both types of growth were encountered in the axillary metastasis and later in recurrent nodules in the postoperative scar.

MALIGNANT SARCOMA IN EMBRYONIC TISSUE

PRODUCED BY BERKEFELD FILTRATES OF THE JENSEN SARCOMA OF RATS *

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The important positive results noted in this paper were reported in brief as early as the spring of 1923.[1] In this paper I will describe my earlier experiments more fully and give others which confirm more completely the previous deductions that cancer is the result, not of a primary change in the cell, but merely of a local change in the environment or in the immediate organization of the tissue containing the cells. The changes in the cell which make it appear as a cancer cell are its reaction to this environment.

The tissue culture has shown definitely that the differentiated cells of the organism can revert to actively growing cells which resemble actively growing cancer cells. In the culture these cells do not grow to form organ structures, but separate one from the other to invade the medium exactly as cancer cells invade the organism.[2]

Appreciating at an early time that these cells were similar in appearance to cancer cells, I removed numbers of them from the cultures and inoculated them into animals, hoping that they would continue their growth as cancer cells. None of these experiments was successful. The cells in every case degenerated and were absorbed in a few days.

It had also been noted in the early experiments that this growth of the cells in the tissue culture can be greatly accelerated, often as much as from twenty to forty times, by the addition of an extract of a young embryo or an extract of a cancer to the medium. The activity of these embryonic and tumor extracts was also undisturbed after they were freed from cells by passage through a Berkefeld filter.

In other of the earlier experiments it became of interest to see, therefore, whether the cells stimulated to grow in the cultures with extracts of embryos and a Berkefeld filtrate of a sarcoma would continue to grow as cancer cells in the organism. Rats were used for these

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1. Burrows, M. T.: The Experimental Production of Malignant Ulcers in Rats, J. Missouri M. A. **20**:145, 1923.

2. Burrows, M. T.: The Growth of Tissues of Chick-Embryos Outside the Body With Special Reference to the Nervous System, J. Exper. Zool. **10**:63, 1911.

experiments. Cultures of the heart muscle and body wall of rat embryos were made by placing fragments of these tissues into a medium composed of 1 part of plasma and of 1 part of either an extract of embryo or of a Berkefeld filtrate of an extract of a Jensen sarcoma of the rat. The extracts were made by grinding equal weights of embryo or sarcoma and isotonic sodium chloride solution in a mortar with white sand. The embryonic extracts were either centrifugalized or allowed to stand until the larger tissue fragments had settled out. The clear supernatant fluid was then used. The sarcoma extracts were treated in the same manner, except that they were freed from cells by passage through a Berkefeld N filter.

In all of these cultures the mesenchyme cells migrate out from the fragments in large numbers and show most active growth and division. These cells in the outer medium are free from all blood vessels and other structures, and appear as a pure mass of growing and dividing cells. The cells from five to ten cultures were carefully collected into a mass and introduced with a needle under the skin of a rat. In each case they degenerated rapidly and were absorbed by the animal in a few days.

A considerable number of these experiments were performed during several years. The continuous failures which accompanied all of them led me at first to believe that these cells were not cancer cells. It was not until the experiments on the general factors regulating growth, differentiation and function of tissue cells had been carried further that I appreciated that this deduction may not be true. These latter studies have shown definitely that differentiated cells can be transformed into growing cells and that the growing cells can be made to differentiate in one case or to function in another simply by changing their immediate environment.[3] The ordinary sequence of change which each cell follows in the normal organism, growth, differentiation and function is something peculiar not to it, but to the changing organization of the whole, which results from its growth. It became evident, therefore, that any change of the proper kind in the organization of the body must revert a functioning cell to a growing cell and that this cell may grow independently. In the cultures, these cells can be made to grow independently and to invade the medium. In the same manner, there was no reason to believe that they cannot grow independently in the body under the influence of the proper change in their environment.

It therefore became of interest to study further the conditions of environment of the cultures suitable for growth in one case, and for

3. Burrows, M. T.: The Tissue Culture in Cancer, Tr. Second Pan. Am. Sc. Cong., Washington, section 8, part 2, 1915, p. 494.

differentiation and function in another. As noted in other papers,[4] these studies have shown that the conditions suitable for an independent growth of the embryonal and cancer cells are simply crowding and stagnation. While crowding and stagnation are necessary for a growth of adult cells, they must be washed first with either plasma or another lipoid solvent, such as benzene.

These factors of crowding and stagnation are important because growth depends on the accumulation to a certain high concentration (S^s) of an actively diffusible substance, the archusia (S).[5] The washing of the adult cells with plasma or other lipoid solvents is important, because the adult tissue contains an excess of a lipoid substance, the ergusia, which inhibits growth when present in high concentrations.[6]

When these facts were applied to the animal organism, it became evident that cancer may be only the result of any condition which removes the excess of growth inhibitor from the tissues, crowds the cells into masses and reduces the blood supply relatively in this mass. The controlling factors for the behavior of cells in the normal organism are those conditions which regulate the proper distribution of blood vessels, cell density and concentration of the growth inhibitor. Cancer is the result not of a primary change in the cell but of a specific change in the environment about the cell.

To cause cancer it was necessary to change not only the cell but also the general organization of the tissue about the cell. To cause a cancerous transformation in embryonic tissue it was necessary to change in a specific manner the normal arrangement of cells and blood vessels in this tissue. It was necessary to crowd the cells into masses and reduce the blood supply to a minimum in these masses. To cause a transfusion of embryonal cells to cancers which can grow in a host it was necessary evidently to change not only the cell but also the organization of fragments of this tissue.

It may not have been surprising, therefore, that the production of a cancer merely by transplanting the tumor cells should have failed. On the other hand, it became evident that if these cells are organized into dense masses in the tissue of the host and if these masses are supplied by a minimum amount of blood, they should grow independently

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4. Burrows, M. T.: The Tissue Culture as a Physiological Method, Tr. Am. Cong. Phys. and Surg. **9**:77, 1913; The Tissue Culture in Vitro, Internat. Cong. Med., Gen. Path. and Path. Anat., London, 1913, p. 217; Energy Production and Transformation in Protoplasm as Seen Through a Study of the Mechanism of Migration and Growth of Body Cells, Am. J. Anat. **37**:289, 1926.

5. Burrows, M. T.: Factors Regulating Cellular Growth and Their Importance in the Explanation of Cancer, South. M. J. **17**:233, 1924.

6. Burrows, M. T., and Jorstad, L. H.: On the Source of Vitamin A in Nature, Am. J. Physiol. **77**:38, 1926.

or become cancers. To cause a cancerous organization in embryonic tissue, it may be necessary only to change the arrangement of blood vessels and cells in the embryonic tissue. While there seemed to be little doubt that these embryonic cells are different from the cancer cells, there is considerable evidence to show that the initiations of growth in the embryonic fragments which had been removed from the embryo might cause these cells to change to true cancer cells. As noted in the foregoing, these embryonal cells had grown independently in the cultures when crowded into a stagnant environment, and they had invaded the medium exactly as cancer cells invade the organism. Later work has only confirmed these deductions.[7]

At various times throughout the study of the behavior of cells in a tissue culture, I had noticed that when fragments of embryonic tissues, such as fragments of skin or heart muscle, are placed in a liquid medium containing an extract of a cancer or of young embryos, the cells often fail to migrate from the fragment, but proliferate actively within it. This proliferation is never uniform in these fragments, but takes place at irregular sites along the periphery. The cells proliferating are either the mesenchyme or the epithelial cells, depending on which are the most numerous and crowded in the fragments. The growing cells do not form any intercellular substances or blood vessels in this proliferation. They proliferate to form dense masses of cells. The other cells and blood vessels of the fragment are slowly destroyed by this proliferation. The microscopic picture in such fragments is that of an actively growing sarcoma or carcinoma.

These changes in the fragments were noted several years ago. At that time I transplanted several such fragments to animals. They always suffered a rapid necrosis and disappeared. In looking into these experiments more carefully in 1921, I appreciated that this necrosis may have been due to the fact that the fragments had been transplanted after the blood vessels had disappeared within them. There is no evidence that cancer cells do not need oxygen for their growth, just as do normal cells. It has been fully shown that the blood vessels of the host do not grow into a transplanted fragment. Grafts become revascularized only through an outward growth of their own blood vessels, which unite with those of the host. The fragments used in the early experiments had been transplanted always after all the blood vessels had been destroyed in them through the local growths of the cells. In these earlier experiments fragments of younger embryos were

7. Burrows, M. T.: The Production of Malignant Ulcers in the Skin of Rats, with Notes on the Cause of Cancer in the Uterus, Breast and Liver; The Nature of Hyalinization and Atrophy in Cells and the Tissues; and, Studies on the Nutrition of Tissue Cells, with a Note on the Treatment and Prevention of Cancer, to be published.

used. The destruction of the vessels and the local growth of cells takes place in the fragments of younger embryos more rapidly than in the older ones.

In repeating these experiments it became of interest, therefore, to use fragments of tissue of slightly older embryos and to transplant them at once after they were treated with the extracts of actively growing tissue. It takes considerable time for any fragment to unite with the host. The idea was to mix the fragments with the tissue extracts and to inject the two together immediately or after a short interval so that the fragments would become changed by the extract within the animal in the interval before the host's vessels grew to unite with those of the fragment. It seemed evident that if one could cause the blood vessels of the fragments to unite with those of the host at a time when they were reduced to that minimum just consistent for the life and growth of the cells, a malignant tumor would develop.

EXPERIMENT

Rats were used for these experiments. With the hope of facilitating the take of the transplants, fragments of embryos which were full brothers and sisters of the hosts were the tissue chosen. For the first experiment, a male and female rat with a litter of nine were purchased. The male was father of the young rats and a brother of the mother. The young rats were born on Jan. 28, 1921. The mother was allowed to continue to suckle the young until they were twenty-eight days old. The male was removed to a separate cage as soon as they were brought to the laboratory. After the litter was weaned, the female was placed with the male for one hour each day. She became impregnated again at 4 p. m., Feb. 28, 1921. At 4 p. m. March 15, 1921, she was killed with ether, and five embryos were removed from the uterus. The embryos were just fifteen days old, not counting the time it takes the eggs to become fertilized after the beginning of copulation. The embryos were immediately chopped into small pieces, 1 mm. or less in diameter, and mixed into 4.5 cc. of a Berkefeld filtrate of a physiologic sodium chloride solution extract of a Jensen sarcoma. In this fluid they remained undisturbed for twenty minutes. After this time 0.75 cc. of the total mixture was injected into the subcutaneous tissue of each of the nine young rats of the former litter. These nine young rats, as stated previously, were full brothers and sisters of the embryos. Only about half of the embryonic material was injected.

In all of these rats, tumors developed rapidly at the site of injection. After sixteen days, one of the tumors was removed. It was composed of embryonic bone, cartilage and several small cysts, lined by either embryonic epidermal or endodermal cells. In this tumor it was noted that the mesenchyme about the bone and cartilage was very cellular. Several parts of it had the general appearance of a sarcoma. Otherwise, the tumor was evidently benign, and the cells were assuming the appearance of adult cells.

At the end of the fourth week, one of the remaining eight tumors was noted to be growing much more rapidly than the others. It had a large posterior part which had become attached to the skin and was soft at its upper pole. The firmer, irregular parts of the benign embryoma could be felt at the upper part.

These parts were definitely attached and continuous with the more posterior smoother and softer portion. In the next six days this tumor doubled its size and it was twice as large as any of the others in the series. It was firmly attached to the skin. At the beginning of the seventh week, the softer end became bluish red and the skin over it commenced to suffer atrophy. At this time the rat was photographed (fig. 1) and then killed with ether. A large grayish, opaque mass, resembling a sarcoma, occupied the whole posterior part of the embryoma. The center of the mass was necrotic and contained disintegrated blood, cartilage and fragments of bone of the embryoma. This tumor was definitely within the capsule of the embryonic mass. It had not invaded the host tissue except where it was attached to the skin. Microscopic examination showed it to be a spindle cell sarcoma. It contained numerous areas of necrosis, and in many

Fig. 1.—Rat posing to show large embryoma in subcutaneous tissue of the chest; in the upper lobulated part of the tumor are the cysts of the benign embryoma; the lower large smooth part is a sarcoma, which is definitely a part of the embryoma.

places its cells had invaded the connective tissue about the skin and intestinal cysts of the experimental embryoma (fig. 2).

Sarcomatous portions of the tumor were transplanted to nineteen young rats; tumors developed rapidly in sixteen, and led to the death of these animals in the course of from two to four months. This tumor was transplanted through nine generations of rats, and it became one of the most malignant transplantable tumors of my collection. It included 100 per cent of the later transplants, which is much more often than the takes peculiar to the Jensen sarcoma from which the filtrate was made. The Jensen tumor seldom takes in more than 50 per cent of the rats.[8]

8. Woglom, W. H.: Regression of the Jensen Sarcoma, J. Cancer Research **9**:171, 1925.

Other malignant tumors did not develop in this series. The embryomas continued to grow for many months, reaching their maximum size at about 9 months. One of the embryomas became attached to the skin at the sixth month. The skin over it showed atrophy, and a small atrophic ulcer developed at the end of the seventh month. A hemorrhage was noted in a cyst in another of the tumors at the sixth month, but malignancy did not develop. Eventually, after from nine months to a year and a half, these animals died or were killed. The tumors in the females that had given birth to and suckled young did not grow as actively, or ceased to grow during the period of gestation. The embryomas of this series resembled otherwise those induced by injecting the untreated embryonic fragment, except that they grew two to three times larger.

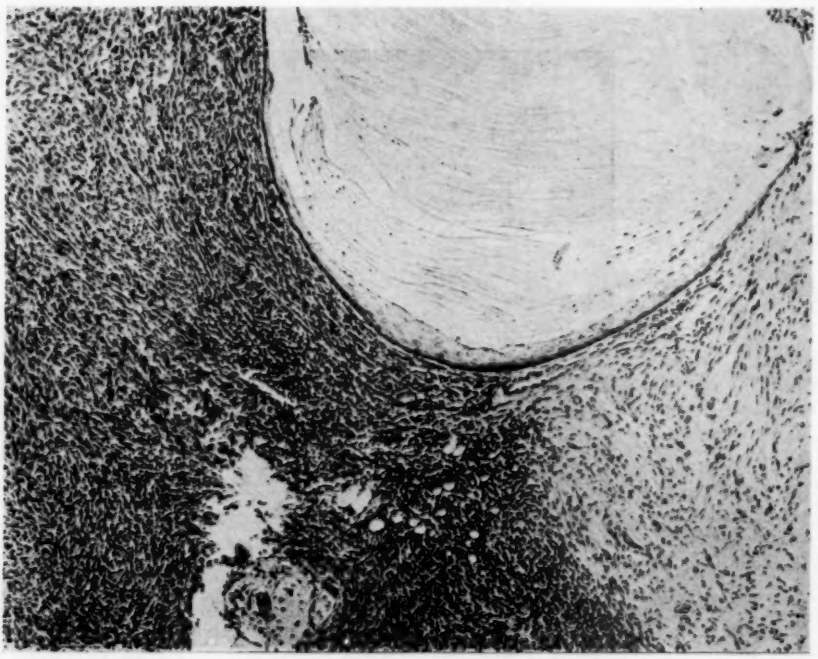

Fig. 2.—Photomicrograph of a section taken through the middle part of the embryoma shown in figure 1; normal embryonic mesenchyme surrounding a cyst lined by squamous epithelium is shown on the right side; the left side of the section is replaced by the actively growing spindle cell sarcoma.

All tumors were eventually removed and on being sectioned showed the characteristic structure of such embryomas in rats. They were lobulated by numerous cysts, which contained a gelatinous, clear, straw colored material or a darker and more viscid jelly-like substance. Between the cysts were masses of cartilage and bone embedded in an embryonic mesenchyme. The cysts for the most part were lined either by a cylindric epithelium, which was thrown into folds and resembled that of the intestines, or by a squamous epithelium which developed typical hair follicles, and sweat and sebaceous glands.

The careful work of many previous authors had shown definitely that when embryos are chopped into small pieces, certain of these pieces survive while others perish. The tissues which survive are the skin and the intestinal epithelium, bone, cartilage, masses of ganglion cells and connective tissue. These tissues grow actively for a time to form benign tumors. In mice these tumors then regress, unless special precautions are taken.[9] In rats, on the other hand, they grow for several months, and undergo normal differentiation. Eventually, as the differentiation is complete, growth ceases, and the tumor persists throughout the life of the rat. A few may regress and others may increase in size, but in most cases, especially when the implantation is made in male rats, the tumors persist as such throughout the life of the animal. In the female, the growth of these tumors is influenced often by pregnancies. The tumors may cease to grow or regress during the pregnancy, only to grow again after the young are born and weaned.

Subsequent to the development of the Cohnheim theory of cancer, which taught that cancer arises from embryonal arrests, many authors studied the effects of the injection of fragments of embryonic tissues in adult animals. They found that this tissue always grew to form benign tumors. These tumors have been termed embryomas or teratomas.[10] Askanazy alone noted the development of a malignant tumor in one of many of these tumors.[11] Malignancy in his case developed late, however, when the tumor was two years old. It resembled the malignant changes which may occur in any benign tumor as the animal nears the end of its normal life. It did not resemble the tumor noted previously, which arose evidently as the result of the action of the tumor extract. This latter tumor evidently commenced its development immediately after the embryonic material was injected. It was a large, well developed sarcoma within six weeks after the tissue was introduced.

Leopold and many others have noticed that the growth of such benign embryomas is proportional to the amount of embryonic material injected and inversely proportional to the age of the embryonic material introduced. When larger amounts of younger embryonic material are introduced the tumors grow larger, but do not show more tendency to become malignant than when older embryonic material is used. The malignant tumor which developed in the foregoing series was evidently related directly to the use of the Berkefeld filtrate of the sarcoma.

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9. Burrows, M. T.: A Preliminary Note on the Study of Experimental Embryomata in Mice, J. Cancer Research **9**:224, 1925.

10. Wolff, Jacob: Lehre von der Krebskrankheit **1**:371, 1907.

11. Askanazy, M.: Die Resultate der experimental Forschung über teratoide Geschwulste, Wien. med. Wchnschr. **2**:2517 and 2577, 1909.

THE JENSEN SARCOMA FILTRATE

The cellfree filtrate of the Jensen sarcoma was used for these experiments because of the importance of determining whether the stimulus for growth in these tumors is only the normal growth stimulus formed by the rat cells concentrated in this region, rather than any stimulus of external origin. Several years ago, Rous [12] found a tumor in a chicken which was readily transplantable to other chickens not only by introducing pieces of it into them, but also by injecting a Berkefeld filtrate of these tumors. This tumor was a sarcoma. The cell free filtrates of it induced sarcoma in other chickens.

Attempts made by many authors to transmit other tumors of chickens, as well as those of other animals, by means of such cellfree filtrates have failed. The experiments with the tissue culture have shown definitely that this failure is not due to the fact that these filtrates do not contain active growth stimulating substances. Filtrates of all malignant tumors stimulate active growth in fixed tissue cells when added to the medium of a tissue culture. The question arises whether the filtrate of the Rous chicken sarcoma is not fundamentally different. Do not these filtrates contain minute formed bodies or bacteria which, of themselves, are able to liberate growth stimulating substances? If the Rous tumor is of this order, one should expect the results obtained with it. The Jensen sarcoma of rats, on the other hand, may be only the result of a primary reduction of the normal growth inhibitor of the body, the ergusia, within and about its cells, of an increase in normal growth stimulus, the archusia, and of a general decrease in its vascular supply. As has already been stated, in this article and elsewhere,[13] any normal cell may grow independently when relieved of the normal growth inhibitor and when it is crowded together with other cells in a stagnant environment. The normal growth stimulus is a water soluble and actively diffusible substance (Wright [14]). It is formed by the cells themselves, and acts to induce growth only in certain high concentrations (S^a). The cells can retain ample of this substance and hold it about themselves only when they are crowded into narrow stagnant confines. In the normal organism the cells contain too much growth inhibitor; they are not sufficiently crowded, and their circulation is too great for them to grow independently. Their growth and normal function is dependent on a supply of growth stimulus, the

12. Rous, P.: A Sarcoma of the Fowl Transmissible by an Agent Separable from the Tumor Cells, J. Exper. Med. **13**:397, 1911.

13. Burrows, M. T.: Studies of the Cause, Prevention and Treatment of Cancer, South. M. J. **19**:279, 1926.

14. Wright, G. P.: On the Dialysability of the Growth-Activating Principle Contained in Extracts of Embryonic Tissues, J. Exper. Med. **43**:591, 1926.

archusia, from without. This is supplied in the form of vitamin B,[15] and it reaches the tissues by way of the blood vessels.

When body cells become crowded into sufficiently stagnant masses suitable for their growth in the body, they continue to grow independently because not only are they able to form and retain ample stimulus for their growth, but also these growing cells destroy the normal tissues and blood vessels about them and continue to maintain the stagnant environment suitable for their growth. This destruction of blood vessels is the result of their high content of growth stimulus, or archusia (S). As noted in other papers,[16] these blood vessels probably persist only so long as the growth stimulus is greater in them than in the tissue cells about them.

If the Jensen sarcoma is of this order, its existence must be dependent entirely on its general organization. If this general organization is disturbed, it should cease to grow and disappear. It should be possible to induce this sarcoma in other animals only by transplanting organized fragments of it into the tissue. When the growth stimulus is extracted and injected into the normal tissues of the animal it should not have any effect on them. It should diffuse readily into the blood vessels and become distributed throughout the animal before it could have any effect on the cells. Such injections should have a general accelerating action on the growth of the animal,[17] rather than any local effect on the cells at the site of injection. On the other hand, if some method were found to hold this stimulus in high concentrations in contact with small areas of these normal cells, they should become cancerous.

It, therefore, became of interest, first, to determine whether the Jensen sarcoma of rats could be transplanted to other animals by means other than the introduction of organized fragments of it into these animals, and, second, to find some means of holding the growth stimulus of this tumor, as well as of that extracted from normal tissues, in contact with small masses of normal tissue: the experiment represents an attempt in this direction. While this experiment is interesting, it has not been proved that the sarcoma which developed in one of the animals used for the experiment did not develop from tumor cells escaping into the sarcoma, or by means other than that deduced in the original hypothesis. To prove the validity of this experiment, one must first prove that the Jensen sarcoma cannot be transplanted by the intro-

15. Burrows, M. T., and Jorstad, L. H.: On the Source of Vitamin B in Nature, J. Physiol. **77**:24, 1926; footnote 6.

16. Burrows, M. T.: Studies on the Nature of the Growth Stimulus in Cancer, J. Cancer Research **10**:239, 1926. Burrows and Jorstad (footnote 6).

17. On several occasions I have noted an excitation of the normal growth of young rats following repeated injections of an extract of a Jensen sarcoma.

duction of a few isolated cells into the host and show that the sarcoma developed only when the organization of the embryonic fragments was changed to the definite degree calculated. According to the hypothesis, sarcoma develops only when the cells are increased per unit area in the fragment and the blood vessels or blood supply is reduced to a certain minimum.

STUDIES ON THE TRANSPLANTATION OF THE JENSEN SARCOMA IN RATS

In the foregoing and in all later experiments, the Berkefeld filtrates of the Jensen sarcoma were prepared by grinding equal weights of the sarcomatous tissue and isotonic sodium chloride solution in a mortar with clean white sand. This extract was then passed through a Berkefeld N filter to remove all of the tumor cells from it. To make certain that these cells were removed, part of a culture of *Bacillus subtilis* was added to each extract at the time of filtration. Subsequent to the filtration, both the remaining extract and the filtrate were cultured as well as examined microscopically for cells. The filtrate used in this experiment and all subsequent filtrates that will be described were free from mobile cells, and cultures of them remained sterile of bacteria.

Only 3.5 cc. of this filtrate was used for the experiment described previously. The remaining 12 cc. was injected into twelve young rats known to be susceptible to fragments of this sarcoma. Reaction was not noted in any of these rats, except that of the needle puncture which healed promptly. In all of the subsequent experiments, each filtrate was tested for bacteria and on animals. Two hundred and fifty rats have received from 1 to 10 cc. of such filtrate, and have not shown any local effects.

In other experiments I filtered extracts of this tumor through thin filter papers. The filtrate in these cases was milky with isolated tumor cells. Fifty rats injected with from 1 to 5 cc. of this fluid also failed to show any reaction to it. Only when numerous small fragments or single larger fragments of this tumor were introduced into the tissues of the host did tumors develop.

FURTHER STUDIES OF THE ACTION OF BERKEFELD FILTRATES OF THE JENSEN SARCOMA OF RAT ON EMBRYONIC TISSUES

The preceding experiments indicate, therefore, that while it is impossible to induce a Jensen sarcoma by introducing a cellfree extract or an extract containing isolated tumor cells into the normal tissues of a rat that has a good vascular supply, it is possible to induce a cancerous organization in an isolated fragment of embryonic tissue that was treated with the filtrate outside the body before it was introduced under the skin of the host. It is interesting to note that not all of the fragments

so treated in the experiment became sarcomas when transplanted into the host; a sarcoma developed only in one part of one of the embryomas that developed in the series of rats used in this experiment.

In going over the rats in this experiment, it was also interesting to note that the benign embryomas grew much more vigorously than is normal in all of the rats of the series except one. The growth of these embryomas was much greater than previously noted when the same quantity of untreated embryomic tissue was introduced into a host under the same conditions. In the one rat, the growth of these tissues was much less than is ordinarily seen.

Fifteen day old rat embryos were used for these experiments, because it was thought that younger embryonic tissue would be destroyed by the filtrate of the Jensen sarcoma and that the cells in older embryonic tissue would suffer little stimulation in contact with the Jensen sarcoma extract. In other papers I have shown that the archusia not only is necessary for the growth of the cells but also is evidently the energy for all other manifestations of life in the cells. When cells are isolated in thin layers of plasma, they become quickly inactive. The archusia (S) cannot concentrate about them. By testing such cells with extracts of tissues rich in archusia (S), it has been found, as noted previously, that the archusia in low concentrations S^1 does not have any effect. In medium concentrations S^2 it causes the cells to migrate and to engorge themselves with protein particles and fat. The cells suffer changes peculiar to their aging in the body in this concentration, and when this concentration of the archusia is localized at one end of a muscle cell it contracts rhythmically. In high concentrations S^3, the cells grow; in all higher concentrations S^4, they suffer a self-digestion. The self-digestion noted in these cells is similar to that suffered by the cells in the central parts of cancerous tissues.

The cancerous tissue contains archusia in S^3 and S^4 concentrations. Embryos of rats twelve and thirteen days old also contain an S^3 concentration of the archusia. This high value decreases with the development of the embryos, and the growth inhibitor increases. The addition of archusia in the form of tumor extract to the medium about fragments of the younger embryos should raise their archusia to a value of S^4 and lead to their rapid destruction. Such is true always for the cells of fragments of 12, 13 and 14 day rat embryos in the culture. The fragments of 15 and 16 day rat embryos do not suffer such a rapid destruction. Their cells, however, are stimulated to grow actively by these filtrates. The cells in fragments of still older embryos respond still less actively.

It was thought, therefore, that the fragments of the younger embryo would not grow if treated with extract of the sarcoma before they were transplanted. They would suffer destruction before they could unite

with the host. It was also thought that the cells in the fragments of 17 and 18 day old embryo would not suffer sufficient stimulation to have any marked effect on their subsequent growth in the organism.

According to the theoretical conditions which lead to the experiments, one should expect sarcomas to develop only in 15 and 16 day old tissue and but few even in these tissues. If the cells in the fragments suffer too great stimulation, the blood vessels must be destroyed; the fragment cannot unite with the host, but must suffer destruction. If the cells are not stimulated to grow sufficiently before the blood vessels of the fragment unite with the host, the general growth of the cells may be excessive for a time, but malignancy should not develop. Malignant tumors should develop only when the cells are increased and the vessels proportionally decreased to a certain definite extent.

These deductions were drawn entirely from the study of the behavior of the tissues in the cultures . It is evident, however, that before any conclusions can be drawn from the experiment cited, it will be necessary to see if the fragments of the younger embryos treated with the Berkefeld filtrate of the Jensen sarcoma failed to grow in the host. It is possible that isolated cells of the sarcoma will fail to grow in the host tissue but will grow when nourished by embryonic tissue. If this is true, one should find a certain number of sarcomas developing in the fragments of the younger embryos, as well as in the older ones.

To draw accurate deductions from the results of these later experiments, it seemed necessary also to study carefully the growth of untreated fragments of embryos of various ages introduced into the host. While, as noted previously, certain laws for the growth of such transplanted tissue have been established, accurate quantitative data are not at hand.

THE GROWTH OF UNTREATED FRAGMENTS OF RAT EMBRYOS IN HOST RATS

Previous authors had noted that the rate of growth and eventual size of the benign embryoma resulting from the transplantation of fragments of embryos into adult hosts are related to the amount of embryonic material introduced, as well as to the age of the embryo. Some of the host rats also were found to be more resistant to the growth of these tumors than others. In the experiment cited, the embryos used were full brothers and sisters of the hosts. In these later experiments, not only was this practice continued, but the parents were also selected from families which were inbred for from one to three generations. This precaution was taken to obviate, as far as possible, any resistance of the hosts for the embryonic cells.

In each instance the host and embryos were reared in the laboratory. Several females were placed with a male. As soon as they were

heavy with young, each was labeled and removed to a separate cage, and the date of the birth of their young was recorded. Any time after the young were 4 weeks old or more, these females were removed to one compartment of a cage that had a central wire partition with a door. The male, father of their previous litter, was left in the other compartment. He could see them but was allowed to mingle with them for only one hour each evening. During this time they were kept under observation. Females taking the male were then removed to his side of the cage and left with him until morning. In the morning the females were returned to their previous litter, which had been weaned. The age of the embryos was calculated always from the beginning of copulation. After the stated time the female was killed with ether; the embryos were removed, immediately cut into pieces about 1 mm. in diameter, and this material injected with a syringe and needle as such, or in a little physiologic sodium chloride solution, into the subcutaneous tissue of the host rats which were their full brothers and sisters of the previous litter. These hosts varied in age from 40 to 55 days.

In the first series of experiments, twelve litters of host rats were injected with single chopped up 13, 14, 15, 16, 17 and 18 day old embryos, respectively. Each embryo removed from the mother was chopped with scissors into pieces about 1 mm. in diameter. One cubic centimeter of sterile physiologic sodium chloride solution was added and the whole sucked into a hypodermic syringe and injected into the subcutaneous tissue of the shoulder of a host. Sixteen hosts were injected with single 13 day rat embryos, fifteen with one 14 day rat embryo, eighteen with one 15 day rat embryo, sixteen with one 16 day embryo, twelve with one 17 day embryo and eleven with one 18 day rat embryo. The 13 day rat embryos weighed 0.039 Gm.; the 14 day embryos, 0.112 Gm.; the 15 day old embryos, 0.168 Gm.; the 16 day old embryo, 0.32 Gm.; the 17 day old embryo, 0.548 Gm., and the 18 day old embryos, 1.0 Gm. Each rat received exactly these quantities of embryonic material. The whole operation was carried on aseptically in a dustproof glass box.

The younger embryonic material grew little or none in many of the rats. Only two tumors of noticeable size developed in the sixteen hosts injected with a single 13 day old rat embryo. Six tumors developed in the fifteen rats injected with one 14 day rat embryo. One of the tumors that developed from the fragments of the 13 day rat embryo and the largest and the smallest tumor that developed from the fragments of the 14 day rat embryos are illustrated in the first and second columns of figure 3. All of the fragments of the 15 day old embryos grew readily. Tumors also developed in all of the rats injected with 16, 17 and 18 day old rat embryos. The largest and smallest of these tumors are shown in the third, fourth, fifth and sixth columns of figure 3. In four of the rats injected with the chopped up 18 day old

embryos, the skin broke down over the embryonic mass, and part of it was discharged. This accounts in part for the small tumors in a few of these rats. The quantity of embryonic material was too great for all of the fragment to take. Some of them suffered autolysis.

These embryomas grew most actively during the first 3 months, but many continued to grow slowly up to 9 months. All of the tumors in figure 3 were removed when they were between 296 and 315 days old.

In a second series of experiments I attempted to ascertain the relation between the size of the embryomas and the amount of embryonic material injected. In one litter of rats, single 13 day rat embryos which were chopped into small pieces were taken up in a small quantity of physiologic sodium chloride solution (0.73 per cent sodium chloride)

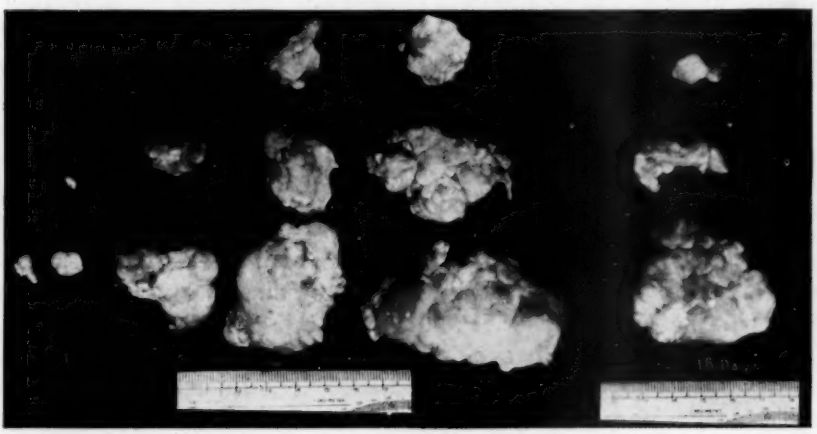

Fig. 3.—Tumors, produced by the injection of untreated fragments of single 13, 14, 15, 16, 17 and 18 day old embryos; the larger and smaller tumors produced by injecting various aged embryos are shown in a part of the verticle columns.

and injected into each host. This experiment was a repetition of the first one of the previous experiments. Small tumors developed in only a few of these hosts. In other cases, the fragments of several embryos were injected. With each increase in the amount of embryonic material the tumor increased slightly in size, but marked increase in the growth was not noticed until 0.3 Gm. of fragments was injected. The first tumor in figure 4 is the outcome after 266 days of the injection of the fragments of a single 13 day old rat embryo. The second tumor is the outcome of the injection of the fragments of eight embryos which were about 13 days old, or 0.264 Gm. of embryonic material. The third tumor weighed 70 Gm. It grew from a mass of fragments of ten embryos slightly less than 13 days old, or 0.3 Gm. of material. For the

production of the fourth tumor, 0.4 Gm. of 13 day old rat embryo fragments was injected. It is practically the same size as the third tumor. In the last two tumors the embryonic tissue increased in bulk over 200 times; 0.34 Gm. yielded a tumor weighing 70 Gm. When the smaller quantity of embryonic tissue was used, such increase was not noted. A tumor weighing only 10 Gm. grew from 0.264 Gm.; it had increased only about 38 times its original weight; 0.034 Gm. of embryonic material increased only to 10 times its original weight.

In the same manner, different quantities of 14, 15, 16 and 17 day old embryos were studied. The growth of the fragments of the 14 day

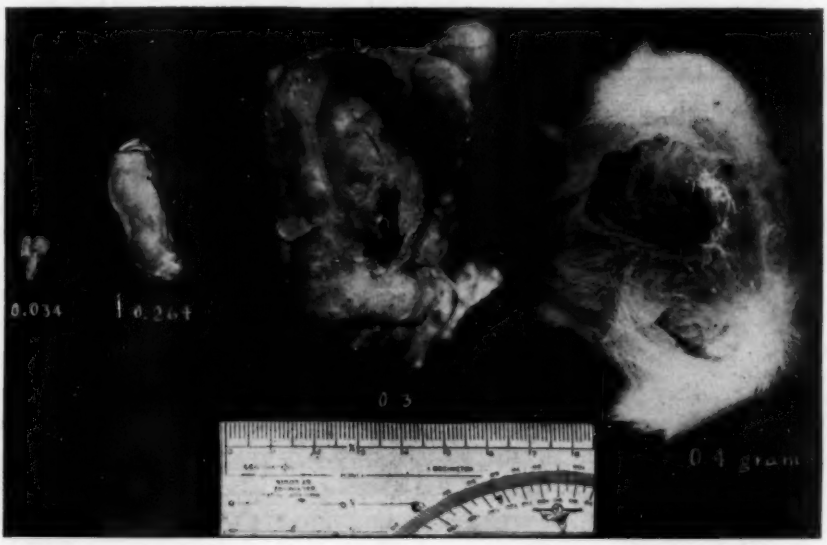

Fig. 4.—Tumors produced by various quantities of chopped up 13 day old rat embryos.

old embryos increased in the same manner as those of the 13 day old embryos, with the increase in the amount of embryonic material injected. This increase in growth was more definitely related to each increase in the amount of embryonic material injected, however, than in the case of the 13 day old embryos. The sudden increase in growth when 0.3 Gm. of material was injected was not observed in this series. As the maximum growth was attained by 0.3 and 0.4 Gm. in the case of the 13 day old embryos, so the maximum also was attained by the same quantity of 14 day old embryos. In the case of the 15 day old embryos, a single embryo often grew as much as three embryos. The maximum growth of the 16 and 17 day old embryos was attained always when the fragments of a single embryo were introduced.

In comparing the relative growth of these tumors, it was interesting to note that the greatest growth was attained always by the younger embryos. No quantity of material from the 14 day old embryos ever produced tumors the size of those produced by the 0.3 and 0.4 Gm. of 13 day old embryos. The largest 14 day old embryonic tumor weighed only 58 Gm. The largest tumor produced by fragments of 15 day old embryos weighed 50 Gm., and the largest tumor of the 16 day old embryos weighed 42 Gm. The largest tumor produced from the fragments of 17 day old embryos reached a weight of 35 Gm., and one from fragments of an 18 day old embryo weighed 25 Gm.

As is evident, therefore, there are two absolute factors in the growth of these tumors, the age of the embryonic tissue and the mass of material injected. When one chopped up 13 day old embryo is injected into a host, the tissue often fails to grow. When growth intervenes, it is never considerable. The same is true when the fragments of one 14 day old embryo are injected. When the pieces from several embryos are injected, growth always intervenes and tumors as large as 70 Gm. may develop.

More than 200 such experiments have been performed. In most instances, when as much as from 0.3 to 0.4 Gm. of tissue was injected, the tumors continued to grow for as long as from five to nine months. After that time, a few of the tumors regressed. Many of them remained large, however, throughout the life of the animal. Located on the shoulder, they were not inconvenient, and the animals did not suffer from them in any case. During pregnancies the growth of the tumor was invariably inhibited, and in many instances regressed. This regression was permanent in some of the animals; in others, the tumor began to grow again after the pregnancies and nursing were interrupted.

While in a few of the 200 animals the tumors were removed at an early time, in most of them they were left undisturbed and the animals were allowed to go through their normal life in the laboratory. These tumors have never become malignant, nor have they shown any tendency toward malignant degeneration.

THE EFFECT OF TUMOR EXTRACT ON THE GROWTH OF FRAGMENTS OF EMBRYONIC TISSUE TRANSPLANTED INTO TISSUES OF YOUNG RATS

When these controls were established, it was possible to study more carefully the effect of Berkefeld filtrates of extracts of the Jensen sarcoma on the transplanted fragments as they had been studied in the first experiment. As noted previously, the most active growth takes place when from 0.3 to 0.4 Gm. of embryonic material is injected. In this series of experiments, I treated 0.4 Gm. of fragments not only of 15 day old embryos, but also of embryos 13, 14, 16 and

17 days of age, with a Berkefeld filtrate of an extract of the Jensen sarcoma. These experiments were performed in the same manner as the first experiment, except that the tissue and extract, especially when fragments of the younger embryos were used, were injected in many cases into the host tissues as soon as they were mixed together. The older tissues were injected any time between five and twenty minutes after they were placed in the tumor filtrate.

As I have already pointed out, the tissues of the younger embryos develop an immediate active growth and an early self-digestion in the tissue culture when placed in the tumor filtrate. The older tissues respond much more slowly. One should expect, therefore, that the fragments of the younger tissue would be destroyed before their blood vessels could unite with the host, while a certain number of the 15 and 16 day old embryos should unite with the host and show a much more active growth or even a sarcomatous transformation.

It was interesting to note that these fragments responded exactly to calculation. As shown in the accompanying table, the growth of all of the fragments of 13 and 14 day old embryos is inhibited. This inhibition is frequently complete. In a few of the animals, benign embryomas developed, but the largest of these weighed only 2 or 3 Gm. A few fragments of skin and bone alone survived. These small tumors stood in striking contrast to the 50 to 70 Gm. tumors of the controls.

Fragments of the 15 day old rat embryos behaved somewhat differently. In ninety-eight of the 107 experiments the growth of the tissue was inhibited. This inhibition was rarely so marked, however, as in the cases of the fragments of the 13 and 14 day old embryos. In practically all the cases, the fragments produced some growth. Small tumors resulted. None of these weighed more than 3 or 4 Gm. In 9 per cent the growth of the fragment was greatly accelerated; the tumor often reached the size of those obtained from injecting untreated fragments of 13 and 14 day old embryos. This stimulation noted in a certain number of the fragments of 15 day old embryos was more noticeable in the series injected with 16 day old embryos. In the series of fragments injected with 16 day old embryos the growth was stimulated in twelve of fifty-two experiments, whereas it was inhibited in the remainder in a manner similar to that seen in the experiments in which fragments of the 15 day old embryos had been injected. Among the stimulated tumors of the 16 day old embryos, another sarcoma developed of the type noted in the first experiment, in which fragments of a 15 day old embryo had been treated and injected.

This sarcoma was similar to the first one described. It was an actively growing spindle cell sarcoma, and it arose definitely within the capsule of the embryomas. In its center was found a mass of cartilage and bone. It invaded a large part of the stroma of the epithelial cysts

of the embryoma and showed the degeneration and hemorrhage in its central part characteristic of sarcomas.

Fragments of this tumor were transplanted to ten young normal rats. These fragments took and grew actively in all of the rats and killed them in from two to four months. Fragments of this second generation were transplanted again to other rats. These took in all the rats inoculated. This tumor was carried through seven generations of rats and was found to maintain its malignant characters. The occurrence of this sarcoma is noted in the table.

Both sarcomas took more readily when transplanted to rats than did the Jensen sarcoma from which the filtrate had been obtained. As Woglom points out, this tumor tends to regress in many of the hosts.[7] The tumors of the experiments also took in the first transplant, as well as in later ones. This is rarely true of sarcomas that have arisen spontaneously in other strains of rats.

This ability to take was not surprising, however, if these tumors had developed, as calculated, from the embryonic mesenchyme, which is

Results of the Study of the Growth of Embryomas from 0.4 Gm. of Fragments of Embryos of Various Ages Treated with a Berkefeld Filtrate of the Jensen Sarcoma

Age of Embryos in Days	Number of Experiments	Number of Inhibitions	Number of Stimulations	Sarcomas
13	21	100	0	0
14	25	100	0	0
15	107	97	10	1
16	52	40	12	1

closely related to the strain of rats in the laboratory. The cells did not need to adapt themselves to new environments, as do the tumor cells from a spontaneous tumor brought from another colony of rats. My colony of a thousand rats had been developed from a few male rats obtained in St. Louis, and from females from the Wistar Institute. Inbreeding had been practiced from the onset of the development of the colony.

The embryonic tissue that suffered stimulation grew rapidly from the onset. The growth of such fragments of 15 and 16 day old embryos grew with the rapidity of fragments of 13 day old embryos. It was interesting to note, however, that these stimulated tumors did not remain at their maximum size, as did the tumors developed from the untreated fragments. They reached their maximum development at from 137 to 180 days. After this time the tumors of the treated fragments, which had reached a size from four to eight times larger than the tumors grown from the untreated fragments, regressed to the size of the untreated ones (fig. 5). As the overgrown tissue in the wound regressed, so these tumors returned to a size which apparently was able

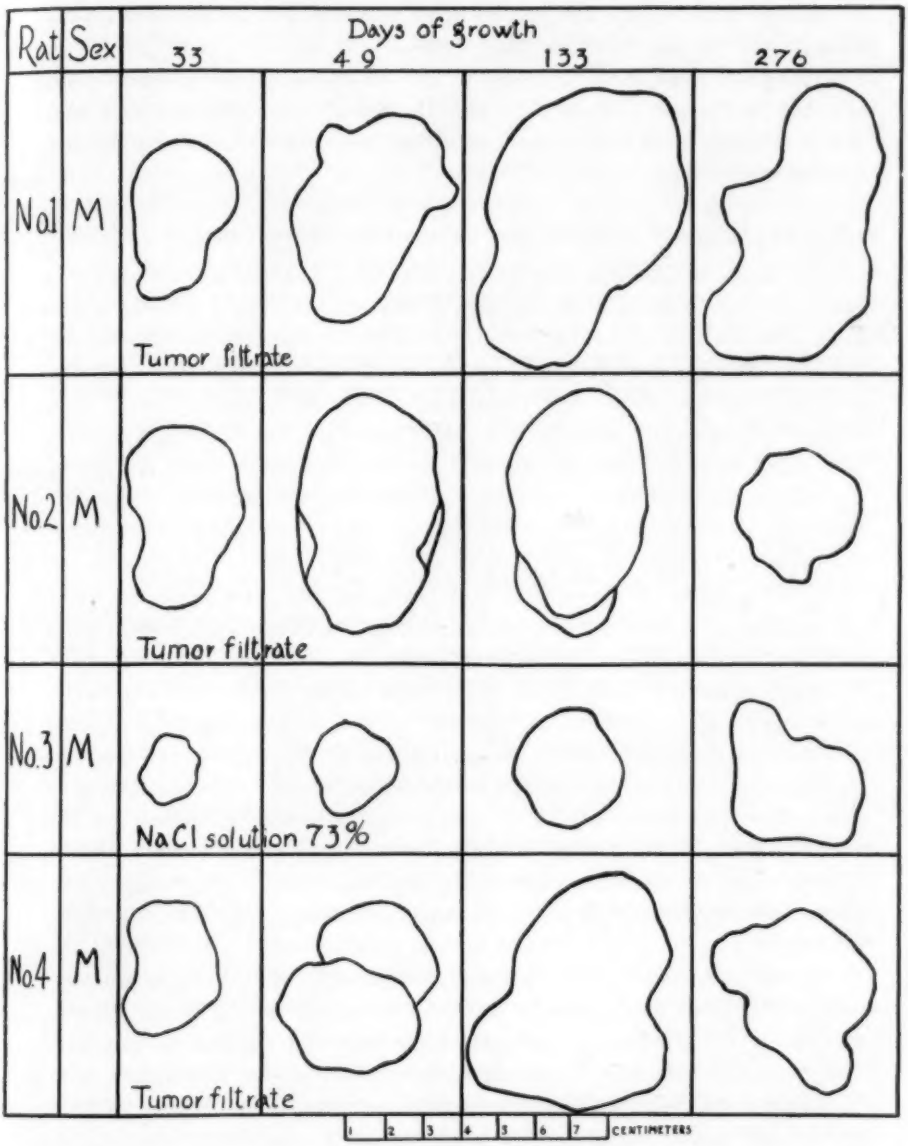

Fig. 5.—Outline drawings of embryomas which were grown from fragments of embryos treated with a Berkefeld filtrate of a Jensen sarcoma, and one that developed from untreated fragments of the same litter of 16 day old rat embryos; the hosts were brothers of the embryos; the growth of the untreated fragments of embryos was progressive, while the treated fragments reached their maximum growth on the hundred and thirty-third day; rats 2 and 4 receded after this time to the size of the control on the two hundred and seventy-sixth day; rat 1 had not decreased so much in size, but had also become smaller after the hundred and thirty-third day.

to balance itself with the rat tissue. The smaller tumors then persisted until the death of the rat.

The growth of the fragments of the 17 day old embryos was little affected by their treatment with the Berkefeld filtrate of the sarcoma. None of them grew noticeably larger than the controls and few showed any evidence of inhibition.

THE EFFECT OF EMBRYONIC EXTRACTS

As noted in the first part of this article, it is possible to transform fragments of embryonic tissue in a tissue culture into a typical sarcomatous-appearing tissue by treatment not only with sarcoma filtrate, but also with extracts of young embryonic tissue. It became of interest in following up these experiments to treat the fragment of 15 and 16 day old embryos with extracts of 12 and 13 day old rat embryos. The extracts were made by grinding the embryos in an equal weight of isotonic sodium chloride solution. Fifty-two experiments were performed. Unfortunately, however, the cells of the younger embryos were not removed from these extracts in only fifteen cases. In all cases in which fragments of tissue were not removed, the growth of the older tissues was inhibited, but not to the extent that these same tissues were inhibited by the action of the sarcoma filtrate.

A more careful analysis of these experiments shows that the variations are to be related to the presence of the younger embryonic cells and not to the extract. The younger embryonic fragments grew actively in these cases and at the expense of the older tissues. The inhibition of the older fragments was due, not to any stimulating action of the extracts, but to an overgrowth of younger embryonal cells. In the fifteen cases in which a clear centrifugalized extract of embryo was used, the older embryonic tissue was stimulated slightly, but never inhibited.

These later experiments led me at first to believe that the stimulus in the embryonic tissue may be different from that of the tumor. Later studies of the differences between cancerous and normal tissues have not given any evidence which need lead one to believe that this is true. The differences between the action of the extracts of cancer and those of normal embryonic tissue are due probably not to a difference in the content of archusia, but to the presence of growth inhibiting substance in the embryonic tissues. The embryonic extracts contain not only the archusia, but also traces of free ergusia, the lipoid substance noted previously. Other experiments have shown that this ergusia evidently owes its origin to the vitamin A of the food. Vitamin B is only the archusia of any actively growing cells in nature. The difference between cancerous and normal tissues is the absence of vitamin A and a high content of vitamin B in the former and a low or high content of vita-

min A and a lower or high vitamin B in the latter. Coal tar acts to produce cancer in that it not only attracts the cells together to form nonvascular masses of them,[18] but removes the ergusia or the growth inhibiting substance from them.[19]

The facts for these deductions are fully discussed in the accompanying papers.[7] As noted at the beginning of this paper, to produce a sarcoma in a normal tissue it is necessary not only to crowd the cells but also to remove the excess of the ergusia from these tissues. This excess was removed in experiments reported in this paper, in which a sarcoma had developed by the overgrowth of a part of the cell of the embryonic fragment. This overgrowth can be produced by any tissue extract containing the archusia. When the tissue extract is free from ergusia, the ergusia necessary for this growth of the cells is taken from the fragment of tissue stimulated. When embryonic extract is used, it is supplied in the extract. When the sarcoma extract is used, it must be taken from the fragment.

THE RELATION BETWEEN THE DEVELOPMENT OF THE SARCOMAS AND THE EMBRYONIC TISSUE TREATED WITH THE FILTRATE

Other experiments with the tissue culture and on animals have indicated that the development of the sarcomas in these cases is to be related not to any peculiarity of the sarcoma filtrate, but to the embryonic tissue treated. Sarcomas, instead of carcinomas, developed in these fragments because the embryonic tissue would not respond otherwise In the body it is well known that sarcomas are peculiar to younger persons, while carcinoma is a disease of adult life.

In none of the experiments so far carried on in the laboratory has it been possible to show that the stimulus for growth in the sarcoma is different from that of the carcinoma, or from that in the growing tissues of the normal organism, such as the growth of the organism and the later and continuous growth of the hair, nail, bone marrow or sex glands. The differences which extracts of these various tissues show are to be related rather to their content of other substances.

There is not any evidence that the Berkefeld filtrate of extracts of the Jensen sarcoma will not stimulate epithelial cells as readily as will the connective tissue. The reason that the sarcomas developed in these embryonic fragments is to be related not to the source of the stimulus used, but to other conditions. As noted in other papers,[20] the ability

18. Jorstad, L. H.: The Behavior of Coal Tar in Adult and Embryonic Tissue, J. Cancer Research **9**:232, 1925.

19. Jorstad, L. H.: The Action of Lipoid Solvents in the Organism and in the Production of Cancer, J. Cancer Research **10**:229, 1926.

20. Burrows, M. T., and Johnston, C. G.: The Action of Oils in the Production of Tumors, Arch. Int. Med. **36**:293 (Sept.) 1925.

of any tissue to respond to stimulation is proportional to its relative cell density. In young embryonic life, the skin is a dense mass of mesenchyme covered by a single or double layer of epithelial cells. In the body, the mesenchyme suffers a continuous development of blood vessels within it, while the epithelial layer remains nonvascular or more stagnant. As development proceeds, the epithelial layer thickens, while the mesenchyme becomes reduced to a mass of intercellular substances and widely separated cells. If fragments of skin of various aged embryos are placed in the tissue culture where both layers become equally stagnant, the mesenchyme of the younger embryos always overgrows the epithelium and destroys it, while in cultures of the skin of older embryos where the epithelium is the more cellular layer, these epithelial cells always become active at the expense of the less densely cellular mesenchyme.

While, as will be noted in a later paper (The Production of Malignant Ulcers in the Skin of Rats, with Notes on the Cause of Cancer in the Uterus, Breast and Liver[7]), other factors probably entered into the production of sarcomas in these particular experiments, in the body it seems evident that the factor of cell density must be most important in determining whether a sarcoma or carcinoma is to develop from any tissue stimulated. In proof of this contention comes the fact that sarcomas arising other than in bone or lymph glands are not common in later life. In the cultures, I have noticed that true sarcoma-like formations developed in fragments of young embryonic skin either spontaneously or after stimulation with embryonic extracts, while Barta,[21] working in this laboratory, found that the ureter of young adult rats treated with embryonic extract in the culture suffers a carcinomatous degeneration. In Barta's experiments, the epithelial layer of the fragments of the ureter invaded the wall of the ureter and the adventitia. The muscle and connective tissue in these cases were reduced by this growth to a hyaline, degenerate mass. The growing epithelial cells not only invaded the surrounding connective tissue and fat of the fragment, but also destroyed the neighboring tissues, formed multinuclear cells and otherwise had all the characters of the invading carcinomatous cells in the organism. Maximow[22] studied the effect of treating fragments of the breast of rabbits with extracts of bone marrow. The bone marrow contains the growth stimulating substance, the archusia, in considerable quantities; it caused typical carcinomatous transformations in these fragments of adult breast tissue.

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21. Barta, E.: Experimental Histological Studies: 1. Some Factors Regulating the Morphology of Tissue (Ureter in Vitro), Anat. Record **29**:33, 1924.

22. Maximow, A.: Ueber krebsähnliche Verwandlung der Milchdruse in Gewebskulture, Virchows Arch. f. path. Anat. **256**:813, 1925.

COMMENT

Since these experiments were performed and described in 1923, Bisceglie [23] has repeated them, using a filtrate of mouse carcinomas. He mixed this filtrate with chopped up chick-embryos, from 2 to 5 days old, injected them into the pectoralis muscle of young chickens, and obtained sarcomas in a certain number of cases. He also performed the same experiments using mouse embryos, instead of chick-embryos, and injected the mixture into mice, but in these experiments he failed to obtain any malignant tumors. He thinks that this failure may have been due to the age of the mouse embryos used. My report of 1923 had already indicated this fact.

While Bisceglie's experiments have not been repeated, they are important, if true in ruling out any possibility of cells in the filtrate being responsible for the development of the tumors, unless these cells are in the form of a parasite.

From the experiments reported in this paper, there is no evidence which indicates that parasites exist in the Berkefeld filtrates of the Jensen sarcoma. The whole action of these filtrates can be explained merely by the fact that they are rich in the normal growth stimulus, the archusia, and poor in the ergusia, or the normal growth inhibitor. This tumor is not similar to the Rous chicken sarcoma.

As will be noted in detail in a later article (The Production of Malignant Ulcers in the Skin of Rats [7]) the change of the embryonic tissue to a sarcoma is not primarily the result of a change in the relative density of cells in the fragment and of a relative reduction in blood supply. The primary change is the decrease in the ergusia in the embryonic tissue.

It is these experiments which have led me to believe that cancer may result not only from substances and conditions that remove the ergusia, or growth inhibitor, from the tissues and crowd the cells in poorly vascularized areas, such as coal tar, and other lipoid solvents, and roentgen rays, radium and other lights, but also from anything that leads to an increased concentration of the archusia at a local site in the tissues, such as chronic inflammatory processes, certain bacteria, certain animal parasites and abnormal physiologic response, functional hyperplasia, etc.[20]

CONCLUSIONS

1. In earlier experiments, actively growing cells from tissue cultures were transplanted to animals, but did not continue their growth.

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23. Bisceglie, V.: Ueber implantationem der mit Tumorfiltrate behandelten Embryonalgewebe und die Genese der Neoplasmen, Ztschr. f. Krebsforsch. **23**:463, 1926.

2. These same cells even when stimulated to grow more actively with a Berkefeld filtrate or a Jensen sarcoma or embryonic extract also failed to grow when transplanted to a host.

3. Sarcomas have developed in animals only when fragments of 15 and 16 day old embryos were treated with a Berkefeld filtrate of an extract of a Jensen sarcoma of rats and transplanted into the host at once or after a short interval.

4. It was not possible to transplant the Jensen sarcoma to other animals, except when fragments of this tumor were introduced. Extracts of isolated cells of the tumor and filtered extracts did not induce tumors.

5. Tumors developed only in those embryonic fragments treated with the Berkefeld filtrate of the Jensen sarcoma. These fragments, as shown by studies with the tissue culture, suffered a specific increase in cells and a decrease in blood vessels, through the stimulating action of this extract.

6. A careful quantitative study of the growth of untreated fragments of embryos introduced into the tissues of a host is included.

7. The experiments showing the effect of embryonic extracts are too few to enable one to draw accurate conclusions. These extracts acted differently, however, from the tumor extract, when the fragments were transplanted to animals. In the cultures, on the other hand, they acted similarly.

8. Evidence was not found to show that the stimulus for growth in carcinoma is different from that in sarcoma. The determining factor in these tumors is the tissue stimulated. Sarcoma developed from the same stimulus in a tissue in which the mesenchyme or connective tissue cells predominated, as carcinoma develops in a tissue in which the epithelial cells predominate.

9. Evidence was not produced to show that the Jensen sarcoma contains other than an increase in the normal growth stimulus of the body and a decrease in the normal growth inhibitor.

EXPERIMENTAL INFARCTION OF THE GLOMERULI IN DOGS

CHRONIC RENAL INSUFFICIENCY *

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AND
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CHICAGO

The anatomic forms of nonsuppurative, bilateral, hematogenous nephritis (table 1) that are most commonly associated with marked clinical manifestations of renal insufficiency are interpreted anatomically, according to modern conceptions, on the basis of a primary change in the glomerulus or in its afferent artery.

In the acute, subacute and chronic forms of glomerular nephritis, the histologic alterations in the glomerulus are chiefly inflammatory. In arteriosclerotic atrophy of the kidneys of Fahr's [1] classification, retrogressive and healing changes occur in the glomerulus, initiated by diminution of the blood supply subsequent to complete or partial occlusion of the lumens of the afferent glomerular arteries by arteriosclerosis. In other words, the degree of renal insufficiency, as a rule, is directly proportional to the number of glomeruli injured. The various microscopic changes of the tubules result not only from diminution of their blood supply that follows partial or complete occlusion of the glomerular tufts, but also from atrophy of disuse, from retrogressive changes due to general metabolic disturbances and from progressive changes, namely, regeneration, healing and compensatory hypertrophy. The apparent increase of interstitial fibrous tissue is chiefly due to approximation of the interstitial stroma after the tubules disappear, and the infiltrations of round cells and the granulation tissue are in large part healing phenomena. The decrease in size of the kidneys, and the depressed and granular regions, so characteristic of the outer surfaces of the chronic forms, result, respectively, from disseminated regions of atrophy of the parenchyma, and from hypertrophy of the remaining tubules.

This theoretical explanation for the essential gross and microscopic alterations of the kidneys included in table 1 is the basis for the experi-

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

* From the Pathologic Laboratories of Rush Medical College of the University of Chicago and the Presbyterian Hospital.

1. Fahr, T.: Pathologische Anatomie des Morbus Brightii, Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1925, vol. 6, p. 167.

mental work described in this preliminary report. It was postulated that the permanent interruption of the circulation through multiple glomerular tufts would initiate the major functional and anatomic alterations of the types of Bright's disease under consideration. This interruption of the glomerular circulation was accomplished by the injection of minute particles of charcoal into the renal arteries. Charcoal was used because its location in the kidneys may easily be determined in microscopic preparations, because it is insoluble in body fluids, thus eliminating direct effects on other organs, and because proliferative changes about the charcoal particles were anticipated.

TABLE 1.—*The Most Common Forms of Chronic Interstitial Nephritis Characterized Clinically by Marked Signs of Renal Insufficiency and by Disturbances in the Cardiovascular System*

	Anatomic Name	Clinical Name
1.	Acute glomerular nephritis.....	Acute hemorrhagic nephritis
2.	Subacute glomerular nephritis, or subacute or chronic diffuse nephritis, large mottled kidneys, and subacute or chronic diffuse nephritis, large pale or fatty kidneys	Subacute or chronic parenchymatous nephritis
3.	Chronic glomerular nephritis, or chronic diffuse nephritis, secondary contracted kidneys	Chronic parenchymatous nephritis
4.	Chronic diffuse nephritis, primary or genuinely contracted kidneys, or malignant arteriosclerotic atrophy of the kidneys	Chronic interstitial nephritis

TECHNIC

Material for Injection.—Charcoal bone,[2] treated with acid, was ground in a mortar, and sieved through a 300 mesh. The dustlike particles were then removed by washing with water. The dustfree charcoal particles were suspended in a 2 per cent acacia emulsion made isotonic with sodium chloride. The acacia emulsion was used to decrease agglutination and sedimentation of the charcoal particles in the injecting apparatus during injection, and in the blood after injection. We usually employed a 2 per cent or 4 per cent charcoal suspension, preferring the former because, presumably, clumping of charcoal particles in the renal arterioles was decreased.

The mixture was injected by means of air pressure. A manometer and rubber-bulb were attached by rubber tubing to the outlet end of a gas washing bottle, and the intake end of the bottle was connected by rubber tubing with a Luer glass adapter that held the injecting needle. All parts, except the manometer and bulb, were wrapped in cloth and sterilized. The autoclaved charcoal suspension was poured into the bottle immediately before injection.

2. Made by J. T. Baker Chemical Company, Phillipsburg, N. J.

Under ether anesthesia, the animal was placed on one side and the lumbar region elevated by a soft pad under the flank. The retroperitoneal approach to the kidney was made through an oblique incision high in the costal-vertebral angle, dividing the external oblique muscle parallel to its fibers and the transversalis and internal oblique transversely.

After the extraperitoneal adipose tissue was separated, the renal artery was easily seen. The kidney was gently pulled ventrally in a plane parallel with the renal artery by an assistant who placed one index finger on either side of the renal pedicle. The kidney rested against the palmar surface about 1 or 2 cm. from the finger-tips, the latter pushing the soft adipose tissue down and thereby making the artery prominent. The adventitial coat was not stripped from the artery because of the consequent vasoconstriction.

A flexible steel needle was used, of 21 or 22 gage, bent at a suitable angle, usually 45 degrees, occasionally even 90 degrees. Penetration of the arterial wall usually could be felt, and was confirmed by the appearance of blood in the glass adapter. The air pressure was maintained at a level so that about 1 cc. of the suspension was injected each minute, the chief merit of the manometer being that it allowed the assistant to maintain a fairly constant pressure. The speed of injection was the most important standard for regulating the pressure. After injection was completed, the needle was withdrawn in a direction exactly parallel with its long axis, to avoid enlarging the perforation of the arterial wall. Bleeding was easily controlled by a tampon in about ten minutes. In only one of about 100 injections was it necessary to ligate the renal artery. Closure was made by suture of the three muscle layers separately, and with interrupted skin sutures. The wounds in almost every instance remained clean, there being only one deep abscess, and stitch abscesses in about 5 per cent of the animals.

In order to secure animal preparations of a chronic type it was found best to inject from 20 to 30 cc. of a 2 per cent suspension in each renal artery. After two or three weeks the amount of renal damage was determined by decrease in the specific gravity of the urine, and the nonprotein nitrogen level of the blood. If there was insufficient change, the animal was then reinjected on one side, provided the kidney was not already small. After two or three weeks, the other kidney was reinjected if the desired degree of renal inefficiency had not yet been attained.

The difficulties encountered in obtaining suitable animal preparations, in order of importance, are as follows:

1. Flaccid paralysis of the hind limbs. This is due to infarction of the spinal cord and occurs most commonly during the injection of the right renal artery. Our explanation of the origin of this compli-

cation has furnished methods for overcoming it, and we believe, therefore, that the explanation is correct. The mouth of the right renal artery, in most dogs, is only a few millimeters cephalad to one of the lumbar arteries, whereas the left is usually caudad. If the charcoal mixture is injected too rapidly into the right renal artery, it is forced into the lumen of the aorta, and carried into the lumbar artery and then to the spinal cord. On the left side, however, the mixture, if forced into the aorta, reaches the center of the blood stream before it passes the level of the next lumbar artery. The following precautions are used: the pressure in the injection apparatus is maintained about 10 mm. of mercury above that which just allows blood to appear in the glass adapter; the color of the renal artery should remain light gray-pink during the injection; the kidney should be pulled away from the aorta only enough to straighten the renal artery; injection is made in the right renal artery first, and after the operation is completed, the animal is allowed to recover from the anesthesia to the stage at which the limbs are moved, in order to determine the presence or absence of paralysis before injection of the left artery. The animals, if paralyzed, usually die in from twenty-four to forty-eight hours after the injection.

2. Total infarction of the kidney from injection of excessive quantities of the charcoal suspension. The correct amount for any batch of ground charcoal is best determined by checking the kidneys with microscopic examination a few days after injection of trial amounts. Attempts were made to estimate the quantity of suspension necessary to plug a few tufts in each glomerulus, the number of particles in each cubic millimeter of the suspension being known, but the results were unsatisfactory. Infarction from thrombosis of the renal artery occurred only once.

3. Double renal artery is encountered about once in thirty operations. It was found impractical to make injections in both arteries, because they usually are too small. These arteries were ligated and the kidneys from the animals, after injection was made into the other kidney, were used for histologic studies.

4. Occasionally the renal artery divides close to the aorta. The point of the needle may protrude into one branch, and as a result the injection reaches only part of the kidney. This can be avoided if the point of the needle is not introduced beyond the field visible to the operator.

GROSS AND MICROSCOPIC ALTERATIONS OF THE KIDNEY

The degree of change in the kidneys is proportionate to the quantity of charcoal injected, and, therefore, only a general division of the gross alterations can be made. With infarction of one-half to three-fourths of the glomeruli, some entirely, others only partially, the following changes occur:

Acute (from one to two weeks).—The kidneys (fig. 1) are uniformly swollen and weigh from 10 to 15 Gm. more than the normal kidneys of a dog of similar size. The surfaces are mottled pale gray-yellow to pale brown to red. The pale brown regions averaging from 1 to 2 mm. in diameter are irregular and surrounded by pale gray-yellow interlacing streaks. The red regions are hemorrhages, usually pinpoint, but occasionally several millimeters in diameter. In surfaces made by sectioning, the cortex is from 2 to 3 mm. wider than normal (fig. 2), the medulla is red to purple from dilated engorged veins, but the striations distinct. The cortex is mottled with irregular pale yellow-brown and light red-brown spots and streaks so that the cortical markings are indistinct.

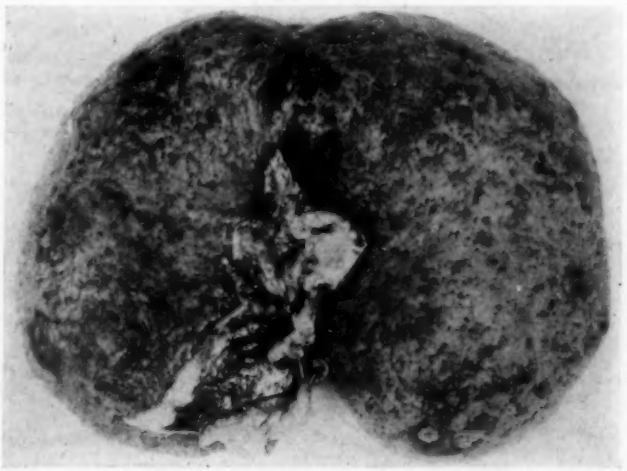

Fig. 1.—Kidney weighing 75 Gm., from a dog that weighed 9.6 Kg.; eleven days before death, it was injected with 35 cc. of a 4 per cent suspension of charcoal; the functional alterations are described in table 2.

The microscopic changes (fig. 3) are chiefly hemorrhage and hyperemia of the glomeruli, retrogressive alterations of the tubules and engorgement of the medullary capillaries and veins.

Approximately 90 per cent of the charcoal particles are in the glomeruli. In one third of the glomeruli multiple charcoal particles are found (from ten to twenty), in another third only a few and in the other third none. The infarcted glomeruli are usually grouped together so that the resulting changes alternate in severity in places about as wide as one low-power field. Hemorrhages and engorgement of the glomerular tufts, necrosis of occasional tufts and a granular amorphous material in the glomerular space are the chief changes here.

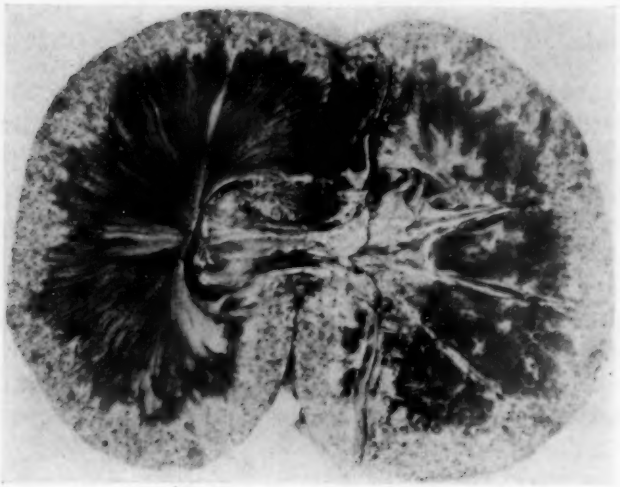

Fig. 2.—Cut surfaces of the kidney in figure 1, illustrating the multiple glomerular hemorrhages, the passive hyperemia of the medulla and the indistinctness of the cortical rays (a thin section was removed from the segment of kidney on the right side).

Fig. 3.—Microscopic preparation from the kidney in figure 1, illustrating extensive but disseminated necrosis of the cortical portions of tubules in a region in which moderate infarction of glomeruli was present; the usual uneven distribution of charcoal particles is seen in the neighboring glomeruli; the interstitial tissue is swollen from edema, hyperemia and hemorrhage.

The outstanding changes are in the tubules. Within a few hours, necrosis of the convoluted tubules is observed. The cells enlarge and obliterate the lumen, the nuclei stain weakly and the cytoplasm stains faintly but uniformly with acid stains. The most marked tubular changes are about the glomeruli that are packed with charcoal particles. Some of the descending loops of Henle also undergo the same changes. The interstitial capillaries everywhere, but especially in the medulla, are engorged with erythrocytes. Occasionally charcoal particles lodge in the capillaries between the tubules in the cortex and the medulla.

Healing Stage.—The kidneys are small (fig. 4). The surface is granular. The elevated portions are light gray-brown, mottled with bright yellow irregular spots, and are separated by darker brown interlacing, depressed regions. In surfaces made by sectioning (fig. 5), the

Fig. 4.—Kidney weighing 17 Gm.; extensive and uniformly disseminated regions of atrophy and compensatory hypertrophy of intervening portions characterize this kidney; the dog lived for three months, and on recovery from a severe period of renal insufficiency an attempt was made at reinjection of the other kidney; death occurred two days later from acute renal insufficiency and infarction of the spinal cord.

cortex is reduced by from 2 to 3 mm. in width, the medulla is about normal width and its striations are retained. The cortical rays are interrupted, and the normal markings indistinct. The glomeruli are indistinct. At the junction with the cortex, there is usually a distinct pale yellow-brown band 2 or 3 mm. wide in the medulla, and, as confirmed by histologic examination, it is the result of retrogressive changes in the descending loops of Henle. A gritty light gray substance is occasionally present in the cortex [3] (fig. 6).

3. It is possible that calcification of necrotic convoluted tubules occurs most frequently if injection is made into only one kidney and if several weeks intervene before injection is made into the other. Perhaps, then, the kidney in which the injection was made is not forced to secrete urine, and calcification may occur more readily during this resting period.

Fig. 5.—In the cut surfaces of the kidney illustrated in figure 4, the cortical markings are indistinct, and the cortex is narrowed much more than the medulla.

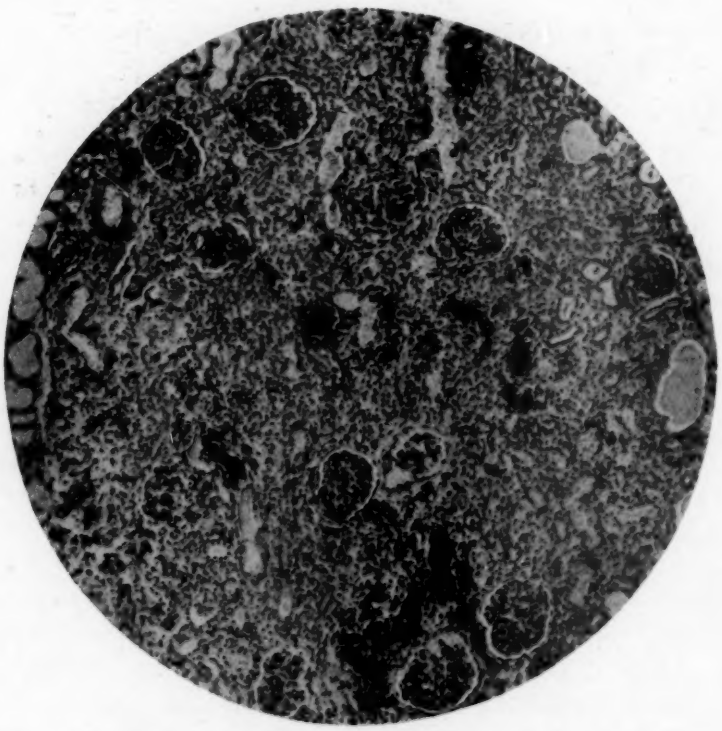

Fig. 6.—Microscopic preparation from a kidney in which injection was performed four weeks before death from renal insufficiency; the black regions are chiefly calcified tubules; some of the glomeruli are atrophic and the capsular spaces obliterated; others have been replaced by scar tissue and the charcoal particles phagocytized by neighboring fixed tissue cells; the majority of convoluted tubules have been destroyed and replaced by approximation of the interstitial tissue; at the sides of the photograph are dilated and hypertrophied convoluted tubules, some of them occluded by hyaline casts.

The microscopic alterations are extremely variable. Commonly, in those regions in which the surface of the kidney is sunken, the glomeruli are in close approximation and are separated by dense cellular fibrous tissue in which occasional tubules still persist. The proximal convoluted tubules that remain frequently stain heavily with basic stains and they are slowly resorbed (fig. 6). The glomeruli are small, and are transformed into round cellular masses (fig. 7), or one or two tufts remain that are unchanged. The glomerular capsule is usually

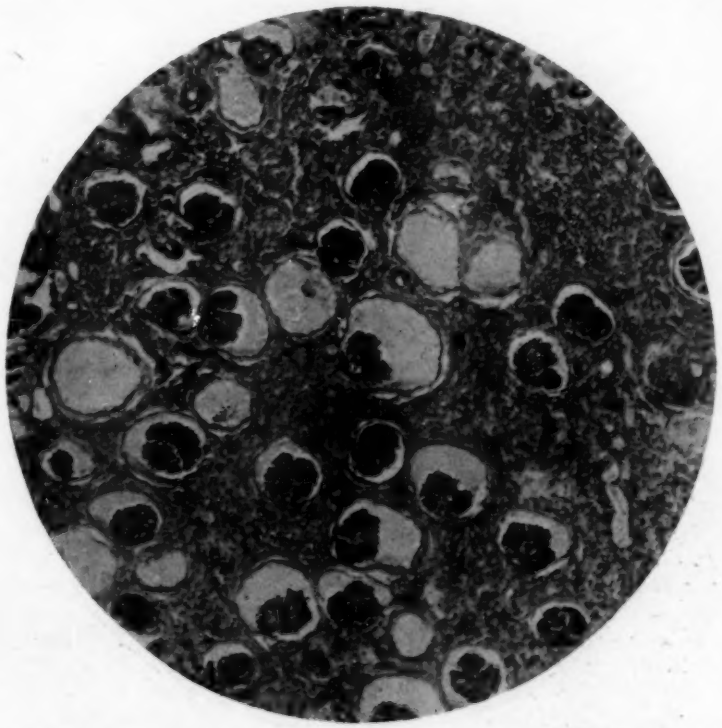

Fig. 7.—Microscopic section through one of the depressed regions of the kidney illustrated in figure 4, in which the tubular portion of the cortex has been resorbed, and the atrophic glomerular tufts are in capsules dilated by retention of urine; the approximation of the glomeruli is characteristic of the contracted forms of chronic interstitial nephritis; hyalinization infrequently follows infarction of glomeruli by charcoal particles.

unchanged. In many tubules casts are seen, and some are denuded of epithelium. The collecting tubules in the medulla are only slightly altered. In some, casts are present. Desquamation of epithelium occurs in those from greatly altered cortical portions. With sudan III preparations, fat is demonstrable in the tubules, in both the cortex and

the medulla, for weeks after injection. The convoluted tubules in those portions in which the glomeruli are slightly altered are characterized by hypertrophy of the epithelium (fig. 8).

The histologic alterations in the older animal preparations have not yet been studied sufficiently to give a general description. Some of the animals, from five to six months since first injection, were in such good physical condition, even with a marked urea and nonprotein nitrogen retention, low phenolsulphonphthalein excretion and low

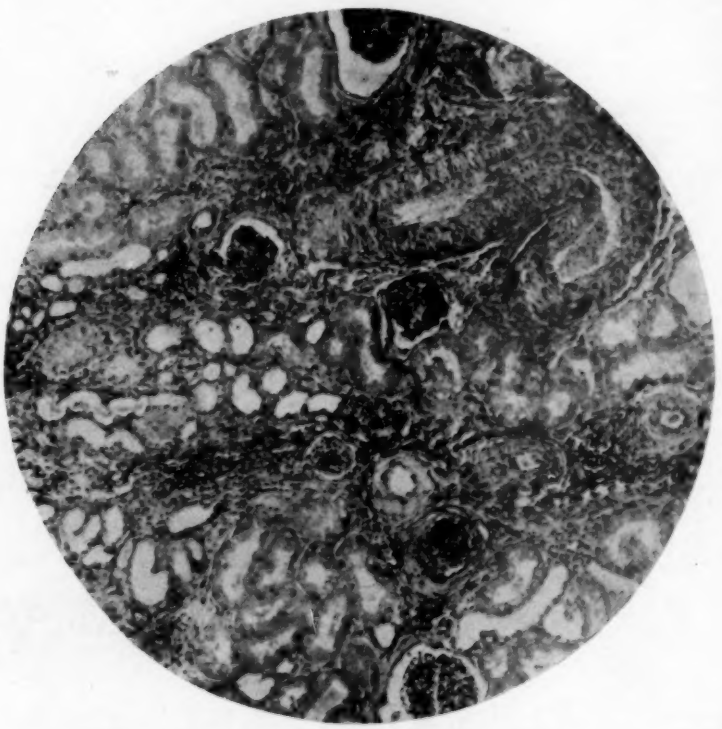

Fig. 8.—Microscopic section through one of the elevated regions in the surface of the kidney illustrated in figure 4, in which hypertrophy and dilatation of convoluted tubules and distortion of some of the loops of Henle are seen; between such regions, atrophy of tubules is observed; in one glomerulus there is hypertrophy of the capsule and partial obliteration of the capsular space by scar tissue.

specific gravity of the urine, that they are being kept for the study of the general gross alterations in the body following long periods of renal insufficiency.

These preliminary studies are concerned chiefly with the development of a method for producing renal insufficiency in dogs, and the anatomic

alterations and the functional disturbances studied include only those that offer a comparison with the more common anatomic and clinical manifestations of chronic interstitial nephritis. When large injections of charcoal were used in strong and healthy dogs, without complete infarction of the kidneys, some died in several days with the alterations described under acute changes. Some dogs recovered from the acute manifestations and entered a chronic stage, which endured for several months in a few; in others gradual improvement occurred so that no noteworthy functional alterations remained. Reinjections have been performed frequently in the latter, thereby securing the best preparations

TABLE 2.—*Important Alterations in Acute Renal Insufficiency in a Young Full-Grown Male Dog*

Date	Weight in Kg.	Water Intake in Cc.	Urine Output in Cc.	Food Intake in Gm., Cooked Beef	Specific Gravity of Urine	Urea Nitrogen in Mg. per 100 Cc. of Blood	Nonprotein Nitrogen in Mg. per 100 Cc. of Blood	Creatinine in Mg. per 100 Cc. of Blood
10/ 6/26	12.6	215	55	350	1.034	.....	.....	....
10/ 7/26	....	305	190	400	1.026	.....	.....	....
10/ 8/26	....	120	230	...	1.014	18.10	38.4	1.4
10/ 8/26	35 cc. of 4 per cent suspension of charcoal injected into right renal artery							
10/ 9/26	Vomiting; no measurements made							
10/10/26	....	470	380	300	1.042	48 hour measurement		
10/12/26	....	250	275	200	1.038	27.7	52.6	1.50
10/13/26	....	275	220	...	1.040	.....	.....	....
10/13/26	Left kidney injected with 25 cc. of 4 per cent suspension of charcoal							
10/14/26	....	Vomiting; quiet; drank about 100 cc. of milk				80.60	101.00	1.8
10/15/26	....	Lethargic; drinks large quantities of water and vomits; not known if urine was passed; no food eaten				134.20	164.00	....
10/16/26	....	About 40 cc. of bloody urine passed						
10/17/26	....	Walks; apprehensive; answers call; stands perfectly still in cage for long periods; diarrhea; vomiting; no urine passed						
10/18/26	9.6	Bloody urine, about 100 cc.; many cellular casts; heavy albumin; anorexia; dog still able to walk; stuporous				172.2	204.10	5.0
10/19/26	....	Dog died; no convulsions.....				307.2	333.4 (antemortum)	10.3

of chronic interstitial nephritis. Some dogs die, following the renal injections, several days after recovery from the operations, without sufficient clinical and anatomic alterations to account for death from renal inefficiency, or from other outstanding diseases such as bronchopneumonia, septicemia or toxemia, the latter measured by retrogressive changes in the important viscera. We have been impressed with the difficulty of interpreting the cause of death in dogs, in some instances, on the basis of anatomic alterations found in the human body. It has been necessary, therefore, to exclude such animals from consideration in connection with renal insufficiency.

The clinical and anatomic alterations of a dog in the acute stage are recorded in table 2. Oliguria, albuminuria, hematuria, low specific gravity and cellular casts constitute the urinary changes. Drowsiness, weakness, anorexia, vomiting and diarrhea are the principal clinical

signs. Generalized clonic convulsions occurred terminally in some instances. Occasionally, the dogs were still willing to walk around and enjoy petting a few hours before spontaneous death.

The clinical alterations of a dog in the chronic stage, and still living on Feb. 22, 1927, are described in table 3. The outstanding characteristics of the chronic group are:

1. Low and fixed specific gravity of the urine. The latter was determined by giving the animals as much fresh raw beef as they could eat for two days, with unrestricted water intake, and then for two days decreasing the latter to one fourth of the average previous daily amount, but maintaining the usual food intake.

2. Polyuria. The increase is usually inversely proportional to the specific gravity, and increases with rise in the nonprotein nitrogen level of the blood.

3. Albuminuria. This is constant, and by the Heller test is represented by a coagulum about 1 mm. wide at the junction of the two fluids

4. Casts. These are constantly present, but in small numbers. They are chiefly hyaline and hyogranular casts.

5. Decrease in phenolsulphonphthalein output. The dye was given intravenously and the urine obtained by catheterization during a two hour period. For months the output in two hours would vary between 10 and 20 per cent.

6. The body weight decreases to about two thirds of preoperative weight.

7. Retention in the blood of urea, nonprotein nitrogen and creatinine. In the animals so far prepared, these substances have averaged the results shown in table 3. Alternate rise and fall are characteristic and are automatically controlled presumably by the dog's appetite, anorexia usually developing simultaneously with increased retention of these nitrogenous substances. We have tried to cause death from renal inefficiency in some of the lively, avaricious dogs by exercise in a revolving drum and indulgence with food, but the only result was a marked increase of the blood nitrogenous substances, followed by anorexia, drowsiness and polyuria until the previous nitrogenous blood level was reached.

8. Hypertension. We have tried to record the blood pressure by bands adapted to the thigh, but the results were so inconsistent in normal animals and in animals on which operation had been performed that no confidence is placed in them. Alterations in the weight of the heart are also difficult to interpret. We have attempted to arrive at a standard for the heart and body weight, but this also is too variable. The weight of the heart is apparently more dependent on the previous activity or muscular development of the dog than on body weight. The variations in thickness of the papillary muscles and trabeculae carnae

TABLE 3.—*Condensed Record of Some of the Alterations in the Urine, Nonprotein Nitrogen Level of the Blood and Water Intake of a Female Mongrel About 2 Years Old, in a Chronic Stage of Renal Insufficiency*

Date	Weight in Kg.	Water Intake in Cc.	Urine Passed in Cc.*	Food Intake in Gm., Cooked Beef	Specific Gravity of Urine	Urea Nitrogen in Mg. per 100 Cc. of Blood	Nonprotein Nitrogen in Mg. per 100 Cc. of Blood	Creatinine in Mg. per 100 Cc. of Blood
8/13/26	15	950	900	...	1.023	.....	.....	....
8/14/26	....	1,050	1,050	...	1.026	.....	.....	....
8/15/26	....	1,230	995	...	1.024	.....	.....	....
8/17/26	....	960	985	No food	1.018	20.20	38.4	1.47
8/17/26	Both kidneys injected with 60 cc. of 2 per cent suspension of charcoal							
8/18/26	Dog vomiting, intake and output not measured							
8/19/26	....	1,500	1,520	400	1.006	26.6	40.40	1.58
8/20/26	....	1,500	1,510	600	1.006	.....	.....	....
8/21/26	....	1,750	1,635	400	1.010	.....	.....	....
8/22/26	....	2,000	1,800	758	1.008	.....	.....	....
8/23/26	14	1,500	2,250	216	1.010	70.90	102.6	1.51
8/24/26	....	2,795	2,200	400	1.010	.....	.....	....
8/25/26	....	2,420	2,225	250	1.005	.....	.....	....
8/26/26	Phenolsulphonphthalein excretion in two hours was 61.7 per cent, about the same as preoperative excretion. The nitrogen blood retention gradually decreased, the quantity of urine decreased, and the specific gravity increased up to September 9, when reinjection was performed in the right kidney							
9/ 5/26	....	900	425	300	1.012	.....	.....	....
9/ 6/26	....	680	800	400	1.018	.....	.....	....
9/ 7/26	....	525	570	425	1.020	.....	.....	....
9/ 8/26	....	830	700	340	1.022	.....	.....	....
9/ 9/26	12.6	475	250	...	1.018	22.4	43.00	1.35
9/10/26	....	640	480	300	1.016	.....	.....	....
9/11/26	....	610	500	250	1.018	.....	.....	....
	Significant alterations did not occur in the blood chemistry and urine, following the reinjection of the right kidney, so on October 7 reinjection was performed in the left kidney							
10/ 5/26	....	1,265	1,275	420	1.014	28.3	45.9	1.35
10/ 6/26	....	1,000	1,170	440	1.014	.....	.....	....
10/ 8/26	....	1,500	1,520	...	1.006	.....	.....	....
10/11/26	....	2,440	2,500	...	1.009	(48 hours measurement)		
10/12/26	....	1,475	1,155	450	1.008	74.60	96.40	2.75
10/13/26	11.2	1,000	1,255	250	1.010	.....	.....	....
10/14/26	....	2,500	(dog vomiting)		.....	71.20	89.70	2.45
10/15/26	....	1,000	1,375	400	1.010	.....	.....	....
10/16/26	....	2,000	1,915	400	1.008	125.80	156.2	3.27
10/17/26	(dog vomiting)							
10/18/26	....	3,400	2,410	200	1.008	73.30	113.60	2.52
	During the next twenty days there was little change. The dog lost its appetite for meat, and on a milk diet gained slightly in weight and was lively, but the nitrogen retention did not go below the level existing on Oct. 18, 1926. During the next few months, the nitrogen retention in the blood, and the polyuria, underwent alternating periods of rise and fall. Short periods are quoted:							
11/13/26	10.4	1,400	1,020	400	1.012	.....	.....	....
11/14/26	....	2,625	1,025	440	1.010	.....	.....	....
11/15/26	....	2,000	2,040	150	1.010	.....	.....	....
11/16/26	....	2,000	1,500	340	1.008	133.3	198.1	2.72
11/17/26	Phenolsulphonphthalein output 14.2 per cent in two hours							
11/18/26	11.1	1,230	1,320	340	1.008	.....	.....	....
11/19/26	....	1,000	980	240	1.006	.....	.....	.....
11/20/26	....	1,090	1,520	300	1.008	.....	.....	....
11/21/26	....	3,110	2,300	...	1.009	.....	.....	....
11/22/26	....	1,075	1,110	250	1.010	83.3	100.5	2.64
12/11/26	....	920	1,100	800	1.010	.....	.....	....
12/13/26	....	4,000	3,900	1,000	1.008	(48 hour measurement)		
12/14/26	....	2,000	2,100	400	1.004	.....	.....	....
12/15/26	10.4	1,420	1,720	500	1.012	142.5	160.0	2.36
12/16/26	....	1,720	1,700	175	1.008	.....	.....	....
12/17/26	....	2,000	1,960	400	1.008	.....	.....	....
12/18/26	....	2,000	2,110	200 Milk	1.008	.....	.....	....
12/23/26	....	1,470	2,400	900 cc.	1.006	149.0	241.5	3.03
12/24/26	....	1,400	2,110	900 cc.	1.008	.....	.....	....
12/25/26	....	2,000	2,370	900 cc.	1.004	.....	.....	....
12/27/26	....	1,700	3,310	1,800 cc.	1.008	(48 hour measurement)		
12/28/26	....	490	.....	900 cc.	(dog vomiting)			
12/29/26	....	300	1,540	900 cc.	1.010	.....	.....	....
12/30/26	....	570	1,160	900 cc.	(dog vomiting)			
12/31/26	....	700	1,520	900 cc.	1.006	126.5	165.5	2.42
1/ 3/27	Phenolsulphonphthalein output in two hours was 18.6 per cent.							

* Occasionally urine was lost for measurement when the dog was exercised, but the resulting errors are not significant.

are also not usable. An electrocardiographic determination of increased left heart preponderance was not feasible.

9. Alterations in the eyegrounds were not observed by Dr. T. D. Allen.

10. Hydrops did not occur.

SUMMARY

A method is described of producing chronic renal insufficiency in dogs by complete or partial infarction of glomeruli. The advantages of this method are as follows:

1. The disturbances in metabolism are dependent primarily on alterations in the kidneys. In many of the other methods of studying experimental chronic nephritis, soluble substances are introduced into the body that may affect many organs.

2. The changes in the kidneys are in many respects similar to those in the common forms of severe chronic nephritis insofar as the general alterations in the glomeruli, tubules and interstitial tissues are concerned. Inflammation is not present, but this could perhaps be simulated to a considerable degree by the use of a slightly soluble irritating substance.

The disadvantages of this method are as follows:

1. The method is cumbersome. The complications are so many that even after the technical procedures are learned, only a few preparations of chronic renal insufficiency are obtained from twenty animals.

2. The extensiveness of the anatomic changes in the kidneys simulates the changes found in chronic nephritis more closely than many of the other methods so far used in the study of chronic renal insufficiency, but the origin is unlike any etiologic factor occurring in human beings, except, perhaps, hemorrhagic embolic nephritis complicating acute thrombo-ulcerative endocarditis, and this is one of the rarer forms of severe chronic interstitial nephritis.

CONGENITAL SYMPATHICOBLASTOMA OF THE SUPRARENAL

REPORT OF A CASE *

OTTO SAPHIR, M.D.
CLEVELAND

Congenital tumors of the suprarenals, variously called neurocytoma, neuroblastoma, sympathicogonioma, etc., are so uncommon that a report of the following case, which is the first of that nature encountered in a series of over 3,950 necropsies, is warranted.

The term sympathicoblastoma is ascribed to the present case because it falls into that subdivision of the classification of nerve tissue tumors recently suggested by Bailey and Cushing.[1]

REPORT OF CASE

Clinical History.—A baby girl, aged 3 weeks, was brought to the hospital, service of Dr. O. Goehle, with the complaint of a markedly enlarged abdomen. The past history revealed that the baby was normally delivered after a full term pregnancy. At birth it was noticed that the skin was peculiarly white. The abdomen gradually became swollen, the stool was grayish white, and shortly before admission a rash appeared on head, shoulders and elbows. The father, mother and three brothers were healthy.

Physical examination showed a markedly distended abdomen with prominent superficial veins. A tumor mass was palpated in the region of the left kidney. The liver was greatly enlarged. The patient died shortly after admission, before a definite clinical diagnosis was made.

The autopsy was made six hours after death. The body was well developed, but undernourished. The skin and sclerae were slightly icteric. The abdomen was distended and the legs were markedly edematous.

The peritoneal cavity contained 200 cc. of a blood tinged fluid. The pericardial and pleural cavities were normal. The lymph nodes throughout were not noticeably enlarged.

Heart and Lungs: Gross pathologic changes were not seen.

Suprarenals: The right suprarenal was normal. The left suprarenal was markedly enlarged (4 by 6 cm. in diameter), surrounded by a fibrous capsule. It compressed the left kidney. The suprarenal was of a soft consistency. On cut section, the entire suprarenal, save the outer portion of the cortex, was replaced by a tumor mass. The tumor was dark mottled red and brown, but showed a few areas of a light gray. The surface of the cut section was finely granular.

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* From the Department of Pathology of Cleveland City Hospital and Western Reserve University School of Medicine.

1. Bailey, Percival, and Cushing, Harvey.: A Classification of the Tumors of the Glioma Group on a Histogenetic Basis with a Correlated Study of Prognosis, Philadelphia, J. B. Lippincott Company, 1926.

Kidneys: The kidneys appeared normal. The upper pole of the left kidney showed a large, smooth, depressed area, apparently produced by the pressure of the suprarenal tumor.

Liver: The liver was markedly enlarged, weighing 650 Gm. The surface was studded with nodules varying in diameter from 5 to 22 mm. On cut section, similar nodules were seen throughout the organ. The smaller nodules were gray, while the larger ones showed a pinkish gray periphery and a red hemorrhagic center, which in many cases was umbilicated. The liver tissue between the nodules was dark green. The architecture was obscured.

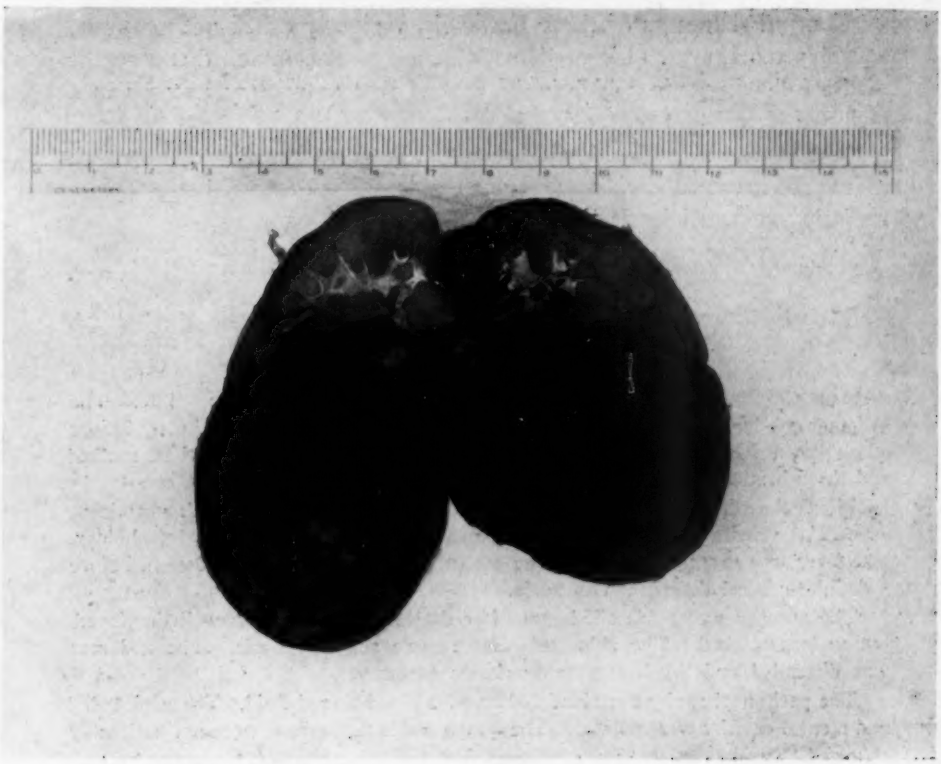

Fig. 1.—Suprarenal tumor and compressed kidney. Scale in centimeters.

The remainder of the organs showed nothing unusual.

Permission for removal of the brain was not obtained.

Histologic Examination: Frozen sections were stained according to Spielmeyer (myelin sheath) and Cajal (unmyelinated nerve fibers). Paraffin sections were stained with hematoxylin and eosin, and according to Bielschowsky (nerve fibers). Specimens fixed in Zenker's fluid were used for Mallory's phosphotungstic stain (neuroglia). The technic of the Cajal stain was taken from Bailey and Cushing, while the details of the other methods were obtained from Mallory and Wright.[2]

2. Mallory, F. B., and Wright, J. H.: Pathological Technic, Philadelphia, W. B. Saunders Company, 1924.

Fig. 2.—Liver, showing numerous metastatic nodules. Scale in centimeters.

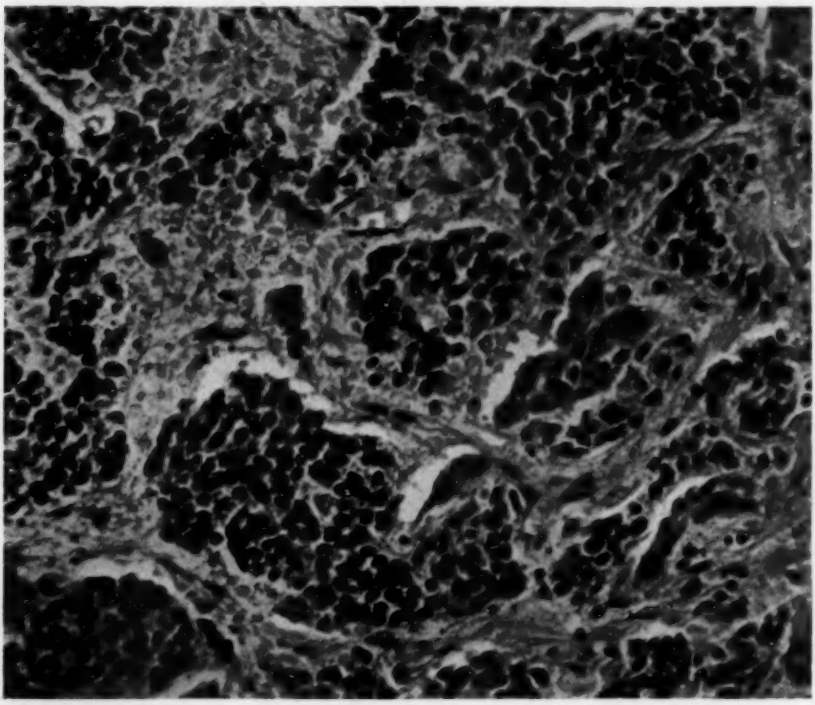

Fig. 3.—Photomicrograph of section of metastasis to liver, showing a pseudo-rosette. Hematoxylin and eosin stains. Objective 8 mm. Ocular 10 X.

Sections stained with hematoxylin and eosin showed that the tumor consisted mainly of round cells, somewhat larger than lymphocytes. The nucleus was deeply stained and details could not be made out. The cytoplasm was hardly visible. Some of the tumor cells were arranged in the form of pseudorosettes, used in the sense of Bailey and Cushing. They differed from true rosettes in not having a central cavity. A comparatively few cells were large and of polyhedral shape. A moderate number of mitotic figures were seen. The stroma contained numerous blood vessels lined by endothelial cells. Vascular slits were not observed. Throughout the sections were found many erythrocytes, outside of vessels. The tumor was confined mainly to the medulla, but infiltrated the cortex to a slight degree. Sections stained for neuroglia showed a few glia fibers throughout the tumor. There was not any evidence of neurofibrillae.

The case here reported shows a primary tumor of the left suprarenal gland with metastasis to the liver. Clinically, the case belongs, according to Ewing,[3] to the Pepper type of so-called "Congenital Sarcoma of Adrenal and Liver in Infants." Histologically, the tumor consists of cells that are similar to those described as nondifferentiated medulloblasts and sympathicoblasts, respectively. Definite neuroblasts or neurofibrillae could not be demonstrated by special stains. A few neuroglia fibers were present, however. The term sympathicoblastoma, therefore, seems appropriate, indicating that most of the tumor cells are nondifferentiated, potential nerve cells.

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3. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1922.

Laboratory Methods and Technical Notes

A RAPID METHOD FOR THE SILVER IMPREGNATION OF RETICULUM*

N. C. Foot, M.D., and M. C. Mènard, B.S., Cincinnati

As the structure and significance of reticulum is receiving more and more attention, the use of a rapid specific staining method becomes essential. The technic of silver impregnation, which is specific for this tissue, has therefore been developed and modified from Bielschowsky's original procedure for demonstrating the axis cylinders of nerves.[1] Maresch [2] gave added impetus to the study of reticulum, following Mall's earlier studies, when he used a modification of this impregnation. Its application to paraffin sections was, however, largely overlooked in this country until recently, when one of us modified it for use on Zenker-fixed material. Heretofore, it had been employed chiefly with frozen sections in practically all laboratories, the more convenient paraffin section technic being neglected in favor of the somewhat laborious and less uniform freezing method.

The original procedure of Maresch has been modified by Foot,[3] Otani [4] and others, as regards slight details, but the length of time required for fully impregnating and counterstaining the sections remains in the neighborhood of from two and a half to three days. While experimenting with the application of the metallic impregnations of del Rio Hortega [5] to Zenker-fixed paraffin sections, as reported in a recent article (Foot [6]), we noticed that the reticulum was usually impregnated along with the nerve tissue; therefore, after some further experimentation, we devised the following modification of the Hortega silver carbonate method, which gives remarkably favorable results with a little practice and due care in the making up of the silver-ammonium carbonate bath.

* From the Department of Pathology, University of Cincinnati College of Medicine.

1. Mallory, F. B., and Wright, J. H.: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924, p. 135.

2. Maresch, R.: Centralbl. f. allg. Pathol. u. path. Anat. **16**:641, 1905.

3. Foot, N. C.: J. Lab. & Clin. Med. **9**:777, 1924. Foot, N. C., and Day, H. A.: Am. J. Path. **1**:431, 1925.

4. Otani, S.: Am. J. Path. **3**:1, 1927.

5. Hortega, del Rio: Tr. d. Lab. de Invest. biol. Univ. de Madrid **15**:367, 1917.

6. Foot, N. C.: A Silver Tannate Technic for Paraffin Sections from the Central Nervous System, Arch. Path. **4**:36 (July) 1927.

In detail, the method is as follows: Sections of Zenker-fixed material are cut to from 5 to 7 microns in paraffin, care being taken to affix them smoothly and flatly to the glass and to dry them out thoroughly in the paraffin oven for at least twelve hours before proceding with the impregnation. The sections are cleared of paraffin in the usual manner, being run through xylol and alcohol of descending percentages into mahogany brown alcoholic iodine, where they stay for five minutes. They are washed and bleached in 5 per cent sodium thiosulphate, washed again and the chromium salts of the Zenker's fluid removed by five minutes' treatment in 0.25 per cent aqueous potassium permanganate and ten minutes' in 5 per cent aqueous oxalic acid, with a rinse between. They are washed first at the tap and then in distilled water.

The sections are next treated with silver-ammonium carbonate, which must be prepared fresh for each batch of slides, as it does not keep well. To 10 cc. of a 10 per cent aqueous solution of silver nitrate, is added 10 cc. of a saturated solution of lithium carbonate, distilled water being used for both. The resulting heavy white precipitate of silver carbonate is allowed to settle, and the supernatant fluid poured off. The precipitate is then washed several times in from 25 to 50 cc. of distilled water, being allowed to settle each time and the supernatant fluid being decanted. After 25 cc. of fresh distilled water is added this washed precipitate is almost dissolved in strong ammonia water, which is added drop by drop while the container is shaken continuously. The precipitate turns grayish brown as the ammonia is added; about 8 to 15 drops of the ammonia will be needed, depending on its concentration. Extreme care must be taken not to overstep the end-point of solution, for it is better to allow a few granules of precipitate to go undissolved. The entire solution is then made up to 100 cc. with distilled water and heated to about 50 C., or until it becomes uncomfortably warm to the touch. The slides are immersed in this solution and placed in an incubator at 37 C. for from ten to fifteen minutes, until they turn a yellowish gray. The temperature of the bath will remain between 40 and 50 C. under these circumstances. Care must be exercised lest the sections become detached from the slides in this warm, alkaline bath; if they have been properly affixed and dried they should not come away.

After being rinsed quickly in distilled water, they are placed in 20 per cent neutral formalin (8 per cent formaldehyde) for two minutes, in which they become dark brown. They are washed well at the tap and transferred to a bath of 1:500 gold chloride in water for two minutes, when they fade to a delicate violet gray. After washing, the superfluous silver salts are removed and the sections fixed in 5 per cent aqueous thiosulphate of soda and then washed. The reticulum is now impregnated and the sections may, if it is desired, be run through ascending percentages of alcohol into xylol and mounted in Canada balsam. To furnish a background, a more lively collagen stain and a more detailed demonstration of the nuclei, a combination of Harris' hematoxylin and van Gieson's stain should be superimposed on the silver impregnation.

(*a*) From three to five minutes in Harris' hematoxylin, wash at tap till blue.

(*b*) Forty-five seconds in van Gieson's picric acid-acid fuchsin stain.

(*c*) Run immediately into 95 per cent alcohol, avoiding water, and follow with absolute alcohol and xylol, mounting in Canada balsam.

The finished, counterstained sections will, as in Foot's modification of the Bielschowsky-Maresch method, show the reticulum in sharply defined black lines, the collagen in vermillion to crimson, the nuclei in reddish brown and the cytoplasm and muscular tissue in yellow. Erythrocytes will vary from yellow to greenish gray. We have tried many variations on the foregoing method, such as changing the length of impregnation time and the concentration of the silver-ammonium carbonate. It was found that as little as 2 cc. of silver nitrate to a like amount of

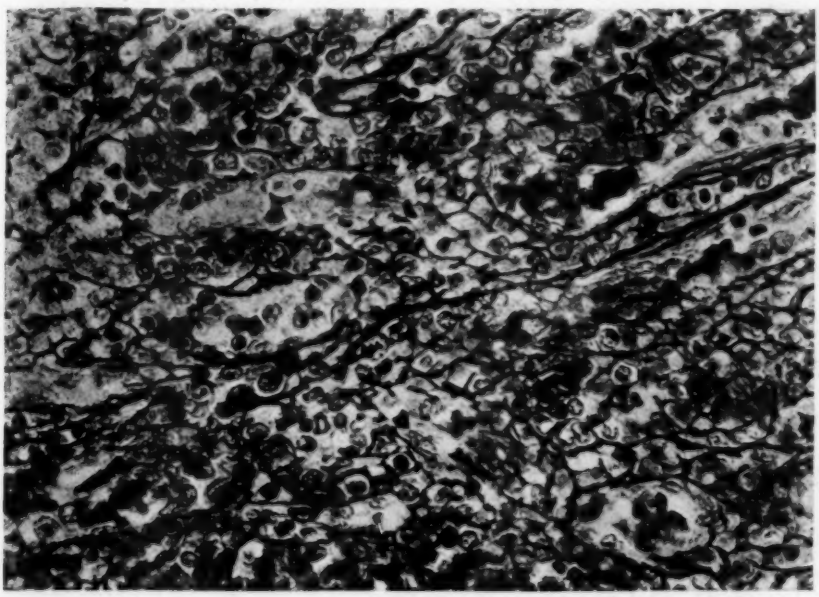
lithium carbonate, diluted to 100 cc. with distilled water, would give excellent results; the most constant and satisfactory impregnations, however, were obtained by the method detailed, which may be briefly summarized as follows:

1. Remove paraffin from sections of Zenker-fixed tissue in the usual way and treat with alcoholic iodine and 5 per cent sodium hyposulphite to remove the mercury salts. Wash at the tap.

2. Treat for five minutes with 0.25 per cent aqueous potassium permanganate and for ten minutes with 5 per cent aqueous oxalic acid, with a wash between. Wash well at tap and follow with a wash in distilled water.

3. Treat for ten or fifteen minutes with silver-ammonium carbonate at about 45 C.

4. Wash in distilled water and reduce in 20 per cent neutral formalin for two minutes. Rinse well at tap.



High power photomicrograph of a section of fairly normal human spleen, at a magnification of about 400 diameters, to show results of the application of the technic herein detailed.

5. Tone in 1:500 gold chloride solution for two minutes.

6. Wash and fix for two minutes in 5 per cent sodium hyposulphite.

7. Wash well and stain for from three to five minutes in Harris' hemotoxylin and then soak in running water till blue.

8. Stain for forty-five seconds in van Gieson's picric acid-acid fuchsin.

9. Wash in 95 per cent alcohol and run through alcohol of ascending percentage into xylol, mounting in Canada balsam.

ADVANTAGES

1. This method shortens the staining time from three days to less than an hour, which makes it valuable when many sections are to be impregnated and a rapid diagnosis on paraffin sections is desired.

2. It is perfectly applicable to paraffin sections from formalin-fixed or Zenker-fixed material, which is a distinct gain when the latter fixation is a part of the laboratory routine.

3. The method is simple and, with a little practice, gives constant results.

4. The reticulin fibers tend to swell in the carbonate bath, which gives them added prominence, in contrast to the Bielschowsky-Maresch method.

As a short, reasonably accurate and reliable method, this technic is valuable for routine preliminary surveys of material supposed to contain reticulum; it will probably prove as satisfactory, in the long run, as the lengthier technic of Bielschowsky-Maresch. This method is not any better than any other, except that it saves time. The figure, for example, demonstrates its value in examination of the human spleen.

General Review

BLOOD GROUPING AND ITS PRACTICAL APPLICATIONS*

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RALEIGH, N. C.

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INTRODUCTION

The opening of the twentieth century witnessed the birth of a problem, the growth of which has kept pace with the years. Blood grouping, originally of clinical importance only, has expanded in its usefulness far beyond its primary limitations, and has invaded the fields of legal medicine, genetics and anthropology. The practical applications of blood grouping may be classed under four heads:

Blood transfusion.
Cases of disputed parentage.
Influence on pathologic, physiologic, or anatomic conditions due to linkage relations or other effects.
Serologic classification of races.

These will be considered in detail.

* From the Genetics Laboratory, N. C. State College.

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BLOOD TRANSFUSION

Historical.—Although the possibilities of blood transfusion were recognized as early as the seventeenth century, and the first authentic transfusion in man was performed by Jean Baptiste Denys in 1667, it was not until this generation that a knowledge of blood groups put transfusion on a sound basis. In fact, it was not until the World War that the application of blood grouping to transfusion became widespread. The story of the progress of blood transfusion from its early inception to the present advanced state of knowledge is fascinating, but I cannot dwell on it at this time. It is important, however, to skim rapidly over the outstanding events until 1900, when blood groups were discovered, and to consider in detail the advances since then.

The success of Denys opened the way for a warm reception of transfusion, but the operations were not always successful, and soon blood transfusion was forbidden in France. Since then the operation has been almost constantly under fire, alternately condemned and approved, as its opponents or proponents have gained the majority.

Not until 1818 did any real advance in transfusion come about, when Blundell revived waning interest in the subject by experiments on dogs, and by the development of a crude apparatus for use in transfusion in man, perhaps the first technic which employed the syringe principle. Bischoff, in 1835, took a great forward step by using defibrinated blood. Coagulation had always been one of the chief obstacles in the way of successful transfusions. The use of defibrinated blood overcame this. Bischoff also suggested that the cells, not the serum or fibrin, were the effective agents.

But at best defibrination was an awkward procedure. Anticoagulants began to be suggested. Ammonia, sodium phosphate, hirudin or leech extract and other substances were tried and abandoned. Salts of oxalic and citric acid were known to be anticoagulants, but were thought to be toxic. In 1914, it was shown by Hustin and by Lewisohn that sodium citrate was an efficient anticoagulant and was not toxic in a dilution which was still effective. This was perhaps the greatest step of all, and the first transfusion of citrated blood by Agote in Buenos Aires in 1914 marked the beginning of the present state of perfection of the operation.

In the meantime, another important discovery had been made. In 1900 Landsteiner and Shattock, working independently, had found that the serum of certain bloods would agglutinate, or clump, the cells of certain other bloods. Even with defibrination, and later with citrate anticoagulation, fatalities in transfusion cases had continued to occur. From the symptoms reported, it is now certain that these were due to incompatibility of bloods. The discovery of the specific iso-agglutinins

in the serum, and the iso-agglutinogens in the red cells, furnished the explanation of these fatalities.

With this brief summary of the historical side of blood transfusion, I shall take it up from the point of view of iso-agglutination and its related phenomena, touching on indications and technic, and discussing in some detail the groups themselves. More complete historical sketches may be found in Keynes (1922), Feinblatt (1926) and Doan (1927).

Iso-Agglutination.—In 1901, Landsteiner classified blood into three groups based on the agglutinating reactions. He called the groups *A*, *B* and *C*. The serum of group *A* agglutinated the corpuscles of another group (*B*), but not those of *A*. The serum of *B* agglutinated the corpuscles of *A*, but not those of *B*. The serum of group *C* agglutinated the corpuscles of both *A* and *B*, but not of *C*. The basis of this scheme was the assumption of two specific iso-agglutinins in the serum and two corresponding agglutinogens in the erythrocytes. The agglutinins and agglutinogens were considered reciprocal, that is, the presence of an agglutinin in a blood precluded the presence of the corresponding agglutinogen, and vice versa.

Decastello and Sturli, in 1902, and Hektoen, in 1907, confirmed these three groups, but noted exceptions, which now form the fourth group. In these exceptions, the serums did not agglutinate any of the corpuscles of the other groups, but the corpuscles of the exceptional bloods were agglutinated by the serums of the other three groups. In 1907, Jansky made a definite classification of the four groups, numbering them 1, 2, 3 and 4. Independently, Moss (1909, 1910) made a classification of the groups in which groups 1 and 4 of Jansky were reversed. Due to the fact that Moss' work was more accessible, this classification has come into wide-spread use in America and parts of Europe. Jansky's work is recognized as having priority rights, however, and has been recommended for adoption. Since use is made of the so-called "universal donor" (group 1 Jansky, group 4 Moss), great confusion has occurred when no statement is made as to whether the Jansky or Moss classification is being used. It seems wise to follow Landsteiner's suggestion to designate the groups by the iso-agglutinogen content of the red cells, i. e., *O*, *A*, *B*, *AB*.

The scheme of the Jansky classification is given in table 1. The correlation of Landsteiner's, Moss' and Jansky's classifications with the proposed new designations is given in table 2.

The proposed new designations, based on the actual agglutinogen content of the corpuscles, will do away with the confusion arising from any system of empiric numbering. Therefore they will be used throughout this discussion, and it is to be hoped that they will be unreservedly incorporated into the literature.

From the interaction of the groups it can be seen that many of the earlier reactions and fatalities were due to agglutination of erythrocytes. Although blood transfusion is perhaps primarily for the purpose of restoring the bulk of the circulating fluid, it is important also in supplying red cells for carrying oxygen. The transfused plasma is so diluted that its agglutinating power for the recipient's cells is relatively unimportant in many cases. Therefore it is sometimes regarded as sufficient that the plasma of the recipient should not agglutinate the corpuscles of the donor. On this basis, group *O* is known as the "universal donor," as the erythrocytes are not agglutinated by any serums. The occasional occurrence of group *O* individuals of especially high titer, however, makes it advisable to select a donor who is of the same group as the recipient. And in any case, a direct

TABLE 1.—*Scheme of the Jansky Classification of Blood Groups*

Group	Effect of Serum	Capacity of Cells
1	Agglutinates cells of 2, 3, 4	Not agglutinated by any serum
2	Agglutinates cells of 3 and 4	Agglutinated by serum of 1 and 3
3	Agglutinates cells of 2 and 4	Agglutinated by serum of 1 and 2
4	Does not agglutinate any cells	Agglutinated by serum of 1, 2, 3

TABLE 2.—*Correlation of the Various Classifications of Blood Groups with the Proposed New Designations*

Landsteiner's Original Classification	Jansky	Moss	Proposed New Designations
O	1	4	O
A	2	2	A
B	3	3	B
—	4	1	AB

matching of bloods immediately preceding transfusion is a necessary precaution, as this eliminates danger from "subgroups," high titers and other exceptions to the "rule of four."

Serologic Basis for the Groups.—The division of human blood into four groups is not so simple as was once thought. Exceptions of two kinds have been presented and elaborated: first, pseudo-agglutination, auto-agglutination, hetero-agglutination, "cold agglutination" and similar phenomena; and second, additions to the four original groups, in the way of subgroups, due to the presence of additional agglutinins and agglutinogens in certain bloods.

The first class of exceptions (pseudo-agglutination and similar phenomena) is important for its scientific interest, and because these conditions may cause mistakes in grouping of blood. Rouleaux formation is one of the commonest pseudo-agglutination phenomena. It was recorded as early as 1853, and has been under discussion ever since. As a source of error in blood typing, it can be eliminated by mechanical

agitation, or, as Lattes (1924) has recently shown, by the addition of lecithin, which deprives serum of its rouleaux-forming properties without affecting the iso-agglutinins. Falgairolle uses three parts of serum to one part of a kaolin suspension (1:3) to prevent pseudo-agglutination. In certain pathologic cases rouleaux formation is marked, and in such cases precautions must be taken against wrong grouping. The use of lecithin or kaolin when any doubt occurs makes this possible.

Sedimentation of corpuscles may simulate massive agglutination. This should not cause mistakes in technic, however, especially in microscopic examination, because the increasing use of the sedimentation test in diagnosis in pregnancy (Fahraeus, 1921; Falgairolle, 1926, and others) and in certain pathologic disturbances has made the characteristics familiar.

Other types of agglutination which may cause mistakes in grouping are auto-agglutination, "cold" agglutination and hetero-agglutination. These may be merely pseudo-agglutination effects resulting from quantitative variations in the properties of the serums or corpuscles, as held by Schiamoff and Jelanski (1923), Lattes and Cavazzuti (1924), Mino (1924) and Lattes (1925), or they may deal with true iso-agglutinins, as held by Clough and Richter (1918), Hirszfeld and Bialosuknia (1922), Chen Pien (1926), Landsteiner and Witt (1926) and others. Probably both types are involved. Certainly in some instances selective absorption tests have indicated the presence of true iso-agglutinins. However, it seems to be agreed by all that under ordinary working conditions these phenomena will not introduce any serious errors into the grouping of bloods. The question cannot rest at this point, however. It is being actively investigated in many laboratories at present, and it must be expected that opinions on these points will be constantly revised in the next few years. So far, it has been clearly demonstrated that auto-agglutination does occasionally occur, that it is considerably influenced by certain pathologic conditions and that there is a "cold"-agglutination which occurs only or mainly at low temperatures and which may be wholly or in part identical with auto-agglutination. The extent to which true iso-agglutinins are involved is as yet an unsolved problem.

The second class of exceptions to the four groups are those included in the so-called "subgroups." These exceptions are based on anomalous reactions of certain serums and cells, and appear to be due to true iso-agglutinins acting in the same way and at the same temperature as those causing the four well known groups. Even here there is considerable difference of opinion, many investigators contending that there are but four groups and no more. The evidence on both sides will be briefly reviewed.

As early as the discovery of blood groups, exceptions have been noted (Landsteiner, 1901; Jansky, 1907). Since then nearly all investigators have noted cases in which serums or cells did not behave as they should. By successive adsorptions with different cells, all of the same group, certain serums were shown to contain several agglutinins within one of the four traditional groups (von Dungern and Hirszfeld, 1911; Schütze, 1921; Vorschutz, 1923; Guthrie and Huck, 1923; Guthrie and Pessel, 1923; Coca and Klein, 1923; Guthrie, Pessel and Huck, 1924; Tebbutt, 1924; Kline, Ecker and Young, 1925; Landsteiner and Witt, 1926; Simpson, 1926; Bunker and Meyers, 1927). Other serums have been noted which gave fewer reactions than were to be expected judging from their group, based on the corpuscles (von Dungern and Hirszfeld, 1911; Meleny and his associates, 1917; Ottenberg, 1911; Sucker, 1924). Correspondingly, erythrocytes have been found to contain agglutinogens not accounted for on the basis of the four known groups.

Guthrie and Huck (1923) were the first to study in a systematic way these exceptions, and to attempt to explain them. They found that the red cells of various persons in group *A* did not always act alike. They adsorbed group *O* serum with erythrocytes of group *AB*. Theoretically, this should have removed all the iso-agglutinins from the group *O* serum. As was to be expected, this adsorbed serum was no longer able to agglutinate cells of group *AB*, *B* and certain of group *A* cells, but contrary to rule it did agglutinate the cells of other members of group *A*. Adsorption of group *O* serum with erythrocytes of the first kind of group *A* left the serum still capable of agglutinating the cells of the second type of group *A*.

Guthrie and Huck deemed it necessary to postulate a third pair of iso-agglutination elements, *C* and *c*, to explain these results. They tentatively estimated the incidence of the new type of group *A* bloods at from 20 to 25 per cent, but later (1924) they questioned this estimate, and raised the incidence. Coca and Klein (1923) found a new pair of iso-agglutination elements in 75 per cent of group *A* bloods (18 out of 23). At first they considered these as different from the new elements found by Guthrie and Huck, because of the increased incidence, but since Guthrie's new estimates it would seem that these are identical. Subsequently, other investigators have found new agglutinable substances in approximately the same percentage of "group *A*" bloods (Landsteiner and Witt, 1924, 76 per cent; Kline, Ecker and Young, 1925, 81 per cent; Simpson, 1926, 81 per cent; Bunker and Meyers, 1927, 78 per cent). Apparently these are all identical, and represent an additional pair of true iso-agglutination elements. The only work so far done on the inheritance of these

additional elements seems to indicate that they are not inherited as mendelian allelomorphs (Coca and Klein, 1923).

Further reports from Guthrie's laboratory (Guthrie and Pessel, 1924; Guthrie, Pessel and Huck, 1924) have indicated the presence of still other agglutinins. None of these have been studied as yet from all angles. Differentiating the antigenic properties of the various anomalous types by means of animal immunization will ultimately help a great deal in clearing up disputed points. Some work has already been done along this line, following the important work on the four known groups by Hooker and Anderson, 1921, and that of Kolmer and Trist in 1920 (Huck and Guthrie, 1924; Schiff and Adelsberger, 1924; Landsteiner, van der Scheer and Witt, 1925; Landsteiner and Witt, 1926; Brahn and Schiff, 1926; Deicher, 1926). Quantitative controls for titers were lacking in the earlier work. These have been furnished by later workers, and have provided one of the chief points in the objection to the postulating of new agglutinins and agglutinogens.

Mino (1924), using quantitative controls, found on repeating Guthrie's work that single adsorptions were not always sufficient to exhaust a specific agglutinin or to satisfy an agglutinogen. Considerable differences in titer of different bloods were found, so that a single adsorption left certain bloods unexhausted, giving differential responses which could be interpreted as indicating additional agglutinating elements, but which really only indicate incomplete adsorption. Other investigators agree that the assumption of additional elements is unnecessary (Lattes and Cavazzuti, 1924; Lattes, 1925; Hübener, 1925; Schiff, 1926; Falgairolle, 1926; Jones and Glynn, 1926). Lattes (1925) explains all of the anomalous results by assuming faulty technic due to low titer, pseudo-agglutination or incomplete adsorption. Tebbutt (1924), after trying cross-agglutination, found a serum like Guthrie's anomalous group *A* serums. He suggests that such cases may be explained as an atrophy of a physiologic quality of the blood serum, or as a congenital condition parallel with a congenital deficiency of some anatomic structure. Bernstein (1925) considers the anomalous cases due to a hereditary partial injury of factor *A*.

Falgairolle (1926) asserts that there are but four groups, with no auto-iso-agglutinins or inter-iso-agglutinins, and that contradictory opinions are based on two sources of error; weak sensitiveness to agglutination, and pseudo-agglutination. Variation in agglutinating power of serums and agglutinophyllic capacity of cells has been demonstrated sufficiently to establish it beyond question (Dyke, 1922; Isaacs, 1924; Price-Jones, 1924; Jones and Glynn, 1926; Freeman and Whitehouse, 1926; Schiff, 1926 and others).

As a sidelight on this question comes the recent work on the inheritance of the groups which places them on the basis of three multiple

allelomorphs (Bernstein, 1925; Furuhata, 1925; Snyder, 1926). This will be discussed fully in the section on medicolegal application, but its bearing on the question under discussion may be briefly considered here. Bernstein suggests that the serums of all groups are identical, that the differences in bloods are in the agglutinogens and that a protective action or equilibrium process of reciprocal binding occurs, which prevents auto-agglutination. In other words, he assumes that the essential reciprocity of agglutinin and agglutinogen in Landsteiner's scheme is only a seeming one. The scheme of agglutinogens and agglutinins in each group according to this hypothesis is given as follows: I shall designate with *a*, *b* and *o* the agglutinins, and retain for the agglutinogens the same designations as for the hereditary factors (*A*, *B*, *O*). According to this conception, there will be present in the serum of each of the six classes *a*, *b* and *o*, with the peculiarity that in each case the agglutinin corresponding to the agglutinogen will be rendered inactive by some process, which may be indicated schematically by parentheses. Moreover, *O* and *o* take a special position, since due to the recessivity of the gene *O* on the one hand, and from as yet unknown causes on the other hand, a direct action of these hypothetical factors is not proved. This is indicated by a superimposed "‿". From the foregoing is constructed the following scheme:

Group	*O*	*A*		*B*		*AB*
Formula	$\breve{O}\breve{O}$	$\overbrace{A\breve{O}}$	$\overbrace{AA}$	$\overbrace{B\breve{O}}$	$\overbrace{BB}$	*AB*
	$ab(\breve{o})$	$(a)b(\breve{o})$	$(a)b\breve{o}$	$a(b)(\breve{o})$	$a(b)\breve{o}$	$(a)(b)\breve{o}$

Landsteiner's scheme is formed from this by the omission of the characters in parentheses and the recessive nonacting *o*.

From the side-chain theory Bernstein postulates that in the tissue cells at the origin of the *a*-agglutinin, for example, so much of the *A*-agglutinogen will be supplied that an equilibrium process of reciprocal binding arises, which protects the red blood corpuscles. Bernstein points out that at the first appearance of the agglutinins in the serum, which takes place later than the appearance of the agglutinogens, apparently spontaneous disarrangements of these internal regulatory processes arise. He advances the fact that the time of the secretions of the agglutinins and convulsions in infancy coincide.

Permanent disarrangements of this process in any individual might cause anomalous reactions in later clinical blood group determinations. The possibilities and implications of this hypothesis are being subjected to experimental attack in the writer's laboratory. Furuhata's three multiple allelomorph hypothesis does not include Bernstein's assumption of the essential similarity of the serums of the four groups.

Whatever may be the ultimate outcome of the question of additional groups, the present uncertain status makes it a practical necessity to

perform direct matching tests immediately before transfusion in addition to determining the groups of donor and recipient.

Technic of Blood Grouping.—Various technics for grouping blood have been worked out, both microscopic and macroscopic. The microscopic methods are to be preferred. The method recommended by the medical section of the National Research Council provides a rapid and accurate technic. For this method test serums of groups *A* and *B* (2 and 3) are necessary. These may be purchased, or they may be prepared from persons of known group and known titer. A drop of group *A* serum is placed on one end of a slide, and a drop of group B serum on the other. Into each serum a drop of cell emulsion is allowed to fall. The slide is tilted and rotated gently so that the cells are uniformly distributed; this is repeated every two minutes. A cover slip is placed on the mixture after two or three minutes of thorough mixing. The slides are examined under the microscope. At room temperature agglutination shows up plainly in from one to ten or fifteen minutes. Irregular clumps of cells, like brick dust, often visible to the naked eye, are the evidences of agglutination.

If neither test serum agglutinates the cells, the blood belongs to group *O* (group 1 Jansky). If only the serum of *B* agglutinates the cells, the blood belongs to group *A*. If the cells are agglutinated only by the group *A* serum, the blood belongs to group *B*. If both serums agglutinate the cells, the blood belongs to group *AB*. The appearance of the cells under the microscope in each of the four groups is shown in figure 1.

The cell emulsion is made by allowing a drop of blood from the finger or ear to fall into a tube containing 3 cc. of isotonic saline solution. If the cells are to be kept more than a few hours before grouping it is best to add 1 per cent citrate to the saline solution and to centrifugalize the cells, pipet off the supernatant fluid, and replace it with pure saline solution before grouping.

This technic will be found satisfactory, although some workers recommend a higher ratio of serum to cells (Wolff, 1924; Gram and Thomasen, 1926, and others). Test serum will retain its agglutinating capacity at least several months if kept sterile. Vincent (1918) found no apparent diminution in the activity of serum one year old which showed a moderate degree of contamination. However, Schumacher and Atzerodt (1926) reported an unfortunate experience which led them to urge the use of fresh serum. They made a test infusion of 5 cc. of blood into a woman of group *O* from her son, also group *O*. The result was an almost fatal collapse. By using fresh test serums, however, the son was found to belong to group *A*. These investigators urge the necessity of checking test serums every two weeks.

Dried serum, once recommended (Sanford, 1918; Hartman, 1918), is not now considered serviceable. Preservatives, also once recommended (Bigger and Wigham, 1922; Vincent, 1918; Karsner, 1918, 1921) are now thought to influence titer (Breitner, 1926), which is important in view of the dispute over pseudo-agglutination. Fresh

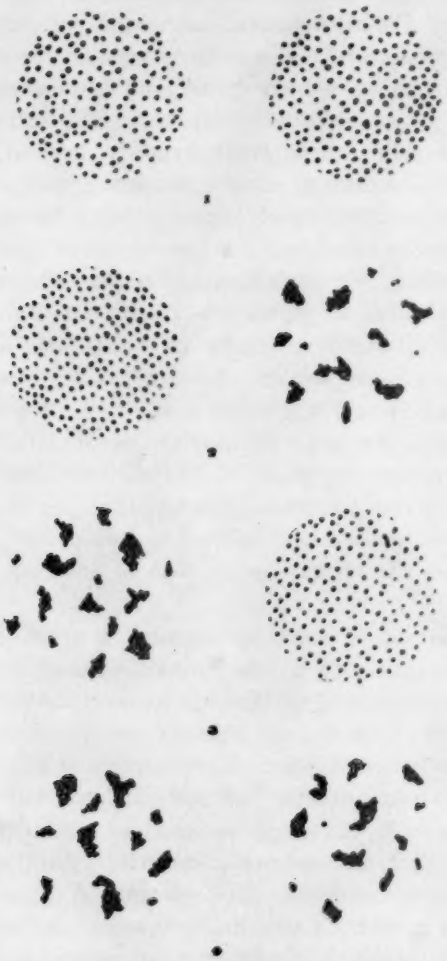

Fig. 1.—Appearance of red cells under the microscope in each of the four blood groups; serum of group *A* on the left, serum of group *B* on the right. Slide 1, group *O;* slide 2, group *A;* slide 3, group *B;* slide 4, group *AB*.

serums, of known high titer, for the group determination of donor and recipient, and direct matching as a precaution against accessory agglutinins and donors of exceptionally high titer, are the preoperative safeguards of transfusion.

Blood Groups in Infants.—In transferring blood into infants, the question arises as to how much of the foregoing routine is necessary. It has long been known that the agglutinophyllic capacity of the cells in usually present at birth, but that the specific agglutinins may not appear until later. Although some have considered that agglutinins are never present in the blood of new-born infants (Schenck, 1905; Bertino, 1906; Cherry and Langrock, 1916; Kimpton, 1918; Baecchi, 1920; De Biasi, 1923; Pollitzer and Rapisardi, 1924), thus making it impossible for any transfused erythrocytes to be agglutinated by the infant's serum, others have shown that agglutinins are sometimes present at birth, and that when they are not, they may be established at any moment during the first two years (Halban, 1900; Langer, 1903; von Dungern, 1910; Titus, 1920; Happ, 1920; Robertson, Brown and Simpson, 1921; Jones, 1921; Unger, 1921; McQuarrie, 1922; Dyke and Budge, 1923; Happ and Zeiler, 1924; du Bouchet, 1926; Falgairolle, 1926; Debré and Hamburger, 1926). Agglutinins have been established in a 7-months fetus (Jones, 1921) and agglutinogens have been observed in fetuses of varying ages, as early as 4 months (von Dungern and Hirszfeld, 1910; Ohnesorge, 1925).

Since iso-agglutinins may occur in the newly born, and the cells nearly always possess their full agglutinophyllic capacity at birth, the same careful procedure that is necessary in the case of adults must be observed in transfusing infants.

Fixity of the Groups.—Is the blood group, once established, fixed for life, or may it be influenced by any known set of conditions? Levine and Segall (1922) reported a temporary change in blood groups after prolonged etherization. Vorschütz (1922) found temporary modifications of the groups after anesthesia, roentgen-ray irradiation and administration of drugs. Harper and Byron (1922) reported a change in the *B*-agglutinogen due to dietary factors. Eden (1922) and Diemer (1922) found changes in the iso-agglutinins after the administration of various drugs (quinine, calcium lactate, antipyrin, arsenic, ether, chloroform) and after exposure to the roentgen ray. Hittmair (1923) reported temporary changes after ether anesthesia. Meyer and Ziskoven (1923) tested these claims, but found no such changes. Siperstein and Kvenberg (1923) likewise found that the agglutinins were not altered after drugs or anesthesia. Huck and Peyton (1923) found no change following anesthesia. Mino (1923) states from experiments with numerous patients and various drugs that it is impossible to modify the iso-agglutinins and change blood grouping. Esposito (1924) attributes the alleged changes to changes of agglomeration. Moldawskaja-Kritschewskaja and Pauli (1925) and Feldmann and Elmanowitsch (1925) found changes in the blood groups of patients with paralysis after malaria treatment. Thomsen (1926) reports the accidental discovery of a transmissible agent whose nature is unknown,

which causes erythrocytes of any group to react as group *AB*. Schumacher and Atzerodt (1926) investigated the alleged changes in blood groups following various procedures, but confirmed only the pseudo-agglutination which occurs after diathermy of the abdomen and after passage of a galvanic current through the body. Hesser (1924), Falgairolle (1926), Schiff (1926), Alperin (1926) and Barsky (1926) affirm the fixity of the groups. It would seem that the blood groups are remarkably stable under rather extreme environmental influences.

Indications for Transfusion.—The clinical indications for transfusion have increased rapidly with the expanding knowledge of the blood groups and the corresponding advance in the safety of the operation. As early as 1872 Leisrink summarized the indications as follows: "Transfusion is indicated in all those pathologic conditions where the blood, in quantity and quality, is so altered that it is unfit to fulfill its physiologic duties." As a generalized statement this still holds true. Keynes (1922) lists the indications under four main heads: (1) acute anemia, (2) anemia of slow onset, (3) hemorrhagic diseases, (4) general toxemia. Feinblatt (1926) enumerates the indications under eleven heads:

(1) Sudden loss of blood from any cause.
(2) Surgical shock.
(3) Illuminating gas poisoning.
(4) Chronic hemorrhagic diseases of the blood.
(5) As a preoperative precaution when the bleeding and clotting time has been found to be delayed.
(6) Blood dyscrasias (pernicious anemia, secondary anemia, leukemia).
(7) Subacute systemic bacterial infections.
(8) Acute septic conditions (typhoid fever, pneumonia, measles, diphtheria, bacteremia, puerperal sepsis and similar conditions).
(9) Diabetic coma.
(10) Debilitated conditions.
(11) Miscellaneous conditions.

Under miscellaneous indications, which have not been thoroughly tested out, Feinblatt recognizes the following: pellagra, nephritis, eclampsia, tropical sprue, acne vulgaris, burns, marasmus and intestinal intoxication of infants, epidemic encephalitis.

Of the indications for transfusion, it is generally conceded that hemorrhage, pernicious anemia and surgical shock are the most important and give the best results under the treatment. In the case of shock, it is necessary that the transfusion be given within a few hours of the onset of shock, otherwise vital centers are likely to be damaged. Transfusion can be considered as a curative measure only in the cases of hemorrhages. In all other cases it is merely palliative, and in some cases it is of doubtful value even in that capacity.

Choosing a Donor.—Lloyd (1926) gives the following criteria for donors. The best donor is a man, from 20 to 30 years old, free from

every kind of disease, and with prominent veins in the antecubital fossa. Women are more inclined to anemia, and the veins are small and apt to be buried in fat. When repeated transfusions are to be made into the same recipient, as in pernicious anemia, for example, it is preferable to use a different donor each time, to prevent anaphylactic reactions.

The health of the donor is exceedingly important, as syphilis (Sydenstricker, Mason and Rivers, 1917), malaria (Van Dijk, 1920; Feinblatt, 1926) and measles (Bauguess, 1924; Harrell, 1924) have been transmitted by transfused blood.

The bloods of respective donors and of the recipient should be grouped, and the donor chosen from the same group as the recipient. If no donor of the same group is available, choose a "universal donor" (group *O*). In addition, as has already been emphasized, direct compatibility tests must be made immediately preceding the operation. Never depend on direct matching alone if it can be avoided, as the results by themselves are not always sufficient guide to the fitness of the donor.

Use of a donor of an incompatible group will cause severe reactions in the recipient. Typical are falling pulse rate, loss of consciousness, hemoglobinuria, pains all over the body, especially in the lumbar region, and heavy and labored breathing. Death usually follows if more than 100 cc. of incompatible blood has been introduced. When less than this amount has been transfused, recovery may occur. The subcutaneous administration of epinephrine (1 cc. of 1 : 1000) and atropine sulphate ($\frac{1}{100}$ grain, or 0.000648 Gm.) is of value in this condition.

Other minor reactions from blood transfusion, not due to choice of donors, are citrate reactions, usually characterized by a chill followed by a rise in temperature of 2 or 3 degrees F. and reactions due to the too rapid introduction of blood, characterized by tightness in the chest and difficult breathing. Multiple embolism leading to sudden death is a rare complication occasionally recorded.

Technic of Transfusion.—Although the methods of blood transfusion, with their modifications and varying forms of apparatus, are legion, there are two main types of procedure: One is direct transfusion, by anastomosis of an artery of the donor with a vein of the recipient; the other is indirect transfusion, in which the blood is drawn from the donor into a vessel or syringe in which clotting is prevented or delayed, and then injected into the recipient's circulation.

Anastomosis of the blood vessels was difficult enough to make transfusion an infrequent occurrence until Crile (1906, 1907) found an effective method. He made the anastomosis by drawing the recipient's vein through a small cannula and cuffing it back. The donor's artery was drawn over the vein and made fast, thus insuring a continuous unbroken passage for the blood. The radial artery at the wrist,

and the median basilic or median cephalic vein at the elbow, are the ones used in this method. Other methods of achieving the same result have been advanced (Elsberg, 1909; Bernheim, 1912, and others), but direct transfusion is not satisfactory for several reasons. It is not possible to measure accurately the amount of blood given; it means that the donor will be permanently scarred and that the artery must be ligatured at the close of the transfusion; it cannot well be done with due regard to the condition of the patient; and, finally, it is an operation involving extremely difficult technic.

Indirect transfusion methods are simpler and preferable. These methods are of two sorts: those in which unaltered whole blood is transfused, and those in which an anticoagulant is added to the blood before it is injected into the recipient. The Kimpton and Brown method (1913), involving the use of a specially prepared paraffin tube, became popular, but offered certain disadvantages, chief of which was the difficulty of completely paraffinizing the tube. The slightest defect in the paraffin coating causes coagulation within the tube. This method, with various modifications (Percy, 1915; Kreuscher, 1918; Schläpfer, 1921; Moll, 1926; Brooks, 1926; Gabriel, 1926), is satisfactory for transfusing unmodified whole blood into adults. The use of unmodified blood is preferable to citrated blood when the indications are to replace diseased with normal blood for the purpose of deriving the benefits of the normal blood as a transplanted tissue (Unger, 1921).

Another set of methods for this purpose are the syringe methods. Since Blundell's use of the syringe in 1818, modifications of syringe technic have gradually been worked out. Lindemann (1913) presented the first really practical technic, involving six Record syringes and two sets of cannulas, three to each set. The syringes are filled with blood from the donor, the blood injected into the recipient, and the syringes washed, in rapid succession. Clotting is not given time to occur in this method. With practice and good assistants, the method is successful. The syringe method, like the others, has been constantly modified (Cooley and Vaughan, 1913; Freund, 1913; Kush, 1915; Bernheim, 1915; Unger, 1915; Abelmann, 1916; Lintz, 1916; Brown, 1918; Graham, 1920; Dorner, 1923; Becart, 1925; Stoll, 1925; Feinblatt, 1925; Carleton, 1926). Unger's modification, involving a two-way stop-cock, has become popular, but does not preclude the possibility of a reflux of blood from recipient to donor. A stream of ether played on the syringe prevents clotting, while the circuits of the recipient and donor are alternately flushed with saline solution.

Feinblatt's instrument consists essentially of two disks, rotating one on the other through an angle of 90 degrees. The proximal disk is perforated to form a channel for communication with a Record syringe attached to it; this opening alternately becomes continuous with either of two similar openings in the peripheral disk, for the inlet and

outlet respectively. The inventor claims for this instrument the reduction of the difficulties of instrumental manipulation to a minimum, and the obviation of the possibility of a reflux of blood from the recipient to the donor.

Methods for indirect transfusion involving anticoagulation have been practically reduced to the various modifications of the citrate method. The suggestion and first applications of sodium citrate have already been mentioned. The credit for the earlier work goes to Hustin (1914), Lewisohn (1915), Agote (1915) and Weil (1915). The citrate forms with the calcium in the blood a soluble double salt, rendering the calcium inert. As blood will not clot without the presence of calcium, the use of citrate effectually prevents coagulation. The citrate methods have simplified technic to such a point that any physician with a knowledge of intravenous therapy can perform the operation satisfactorily; they have obviated the danger of clotting; they have done away with the necessity for special apparatus; they have made possible a wide range in the time and distance of transfusion, and they have reduced to a large extent pain, excitement and fear on the part of donor and recipient. The disadvantages are citrate reactions, already mentioned, and the fact that the availability of the methods may lead to the performance of the operation without sufficient experience. There seems to be a variation in the tolerance of different people to sodium citrate. It has been stated that citrate renders the corpuscles more fragile, diminishes complement in the donor's serum, reduces the opsonic index of the plasma and the phagocytic power of the leukocytes, and destroys platelets (Drinker and Brittingham, 1919; Unger, 1921; Colebrook and Storer, 1923; Agnew, 1924; Thornton, 1925). Mellon, Hastings and Casey (1922) were not able to confirm Unger's statements concerning the destructive influence of citrate on phagocytic activity, opsonins and complement.

The citrate method consists essentially of drawing blood from the donor into a glass receptacle containing a 2.5 per cent solution of sodium citrate (50 cc. of the citrate solution for 450 cc. of blood), and injecting the citrated blood into the recipient. Modifications of the technic have involved closed receptacles for collecting the blood, the addition of citrate under aseptic conditions, variation in the strength of the citrate, the use of pressure other than gravity for injecting the citrated blood and the extension of time between the collecting and injecting of the blood (Rous and Turner, 1916; Robertson, 1918; Hunt, 1918; Hoffman and Habein, 1921; Hartman, 1922; Bigger and Wigham, 1922; Cowles and Antz, 1923; Torii, 1923; Dutton, 1924; Kolmer, 1924; Gibson, 1926; Desplas and Peyre, 1926; Benda and Le Clerc, 1926).

The usual dosage is 500 cc., but it may vary from 300 to 750 or even 1,000 cc. at the discretion of the operator, depending on the age,

weight and clinical condition of the patient. In children the dosage may vary from 50 to 150 cc.

Doan (1927) lists the possible routes by which blood may be given to the recipient as follows: intravenous, the usual method of choice; intra-arterial, which obviates the danger of overdistention of the right side of the heart; subcutaneous, intramuscular, or intragluteal, infrequently employed; into the longitudinal sinus in infants; intraperitoneal, usually in children; by rectum, in cases of large hemorrhage in an infected field, as a rectal drip; via the corpus cavernosum, in moribund, arteriosclerotic, elderly patients; intracardiac in desperate cases.

Other Considerations.—In the future, white blood cells and platelets may have to be considered in addition to red cell agglutination in cases of transfusion. On the basis of a study of the effect of serums on white cells, Doan (1926) found that the serums of certain persons exerted a marked toxic effect on the white blood cells of certain others. He suggests the direct matching of donor and recipient for white blood cell compatibility in addition to the other tests. Studies of the platelets in regard to group agglutination have so far given negative results (Toda, 1923), but the question is worthy of further study (Doan, 1927).

Isohemolysins apparently need not enter into the practical application of blood grouping to transfusion, as isohemolysis is practically always preceded or accompanied by iso-agglutination. Kolmer (1924) believes that isohemolysins may occur in serums which do not contain iso-agglutinins, and states that in cases of carcinoma, the serum of the patient may be hemolytic and result in intravascular hemolysis, despite careful preliminary tests. However, in ordinary compatibility tests, isohemolysins are not considered. If they are to be looked for, macroscopic technic would be better than the slide technic.

Studies on the length of life of transfused cells have indicated that they may live as long as four months. but various studies give widely varying results, and no entirely satisfactory technic for this problem has been devised. However, there can be no doubt that functional oxygen carriers are added to a blood by proper transfusion, and that this is one of the benefits of transfusion, along with increase in bulk of the circulating fluid, and the hematopoietic stimulus.

Transfusion, then, is the first and most important application of blood grouping. Whether transfusion is to find wider and wider use, or whether it is to be restricted by the expanding knowledge of the fundamental causes of the conditions now calling for it, is a question for the future. Feinblatt (1926) states:

> It is to be hoped that, in the near future, the medical mind will direct its attention to a more thorough crystallization of the indications for blood transfusion and to an extension of the field of usefulness of this therapeutic measure.

Doan (1927) goes a step farther and says:

> The work of the recent past and the present on the physiology of the blood and connective tissues gives real promise for the future in the way of a control of the blood which shall mean eventually less and less dependence on transfusion as a palliative measure.

CASES OF DISPUTED PARENTAGE

Inheritance of the Groups.—Occurring as fixed biochemical entities, the four blood groups have been subject to statistical study for years. In 1908 Ottenberg and Epstein made the first suggestion that the groups might be subject to mendelian inheritance. The first experimental study of this possibility was made in 1910 by von Dungern and Hirszfeld. They studied the blood in two generations of each of seventy-two families, comprising in all 348 individuals. From this they concluded that the blood groups were inherited as two independent pairs of mendelian factors, the iso-agglutinogens (*A* and *B*) being dominant to their respective iso-agglutinins (*a* and *b*). On this basis, a specific agglutinogen could not appear in a child unless it was present in one of the parents. This fundamental fact has never been seriously questioned to the present day, although the assumption of two independent pairs of factors is no longer warranted.

Von Dungern and Hirszfeld's conclusions were confirmed and upheld by many later workers (Learmonth, 1920; Weszeczky, 1920; Ottenberg, 1921, 1922, 1923; Keynes, 1922; Tebbutt and McConnel, 1922; Jervell, 1923; Dyke and Budge, 1923; Kirihara, 1924; Plüss, 1924; Mino, 1924; Hirszfeld, Hirszfeld and Brokman, 1924; Dossena, 1924; Avdieva and Grizevicz, 1925; Staquet, 1926). Buchanan (1922, 1923) took exception to the inheritance conclusions, and especially to their application on a medicolegal basis, which was then being advanced by Ottenberg. Buchanan based his objections on several family histories which gave results not in accord with the mendelian expectation. Buchanan's discussions of his results show such a lack of familiarity with the laws of inheritance and their application that his objections have never been taken seriously, and the weaknesses in his arguments have several times been pointed out (Ottenberg, 1922, 1923; Keynes, 1922; Gichner, 1922). The medicolegal application is based on the simple fact, well established, that an agglutinogen never appears in a child unless it was present in one of the parents. The cases in which an agglutinogen not present in one of the parents has appeared in a child (Learmonth, 1920; Buchanan, 1923; Mino, 1924) present three possibilities: (1) the mendelian laws do not apply to the blood groups; (2) the alleged parentage is incorrect; (3) there has been a mistake in technic or observation. These exceptions are so rare that they do not invalidate the assumption that the groups are subject to simple mendelian

inheritance. This leaves two explanations for such anomalous cases: illegitimacy or mistakes in technic.

The necessity claimed by Buchanan of knowing three generations in every family studied does not exist. For the cases in which one parent can be identified as to group by knowing the groups of the child and the other parent, two generations are sufficient.

In 1925 it was suggested independently by Bernstein in Germany and Furuhata in Japan that the inheritance results could be better explained by assuming the inherited factors to be a series of three multiple allelomorphs rather than two independent pairs of factors. This was immediately confirmed with actual family histories by Snyder (1926) in this country. Two hundred families, comprising 1,095 individuals, were studied, and the results were treated statistically with the results of all the inheritance studies to date. It was concluded that the hypothesis of two independent pairs of factors would have to be discarded, and in its place the newer hypothesis of triple allelomorphs

TABLE 3.—*Genetic Formulas of the Four Blood Groups*

Group	Two Independent Pairs of Factors	Three Multiple Allelomorphs
O	aabb	OO
A	AAbb, Aabb	AA, AO
B	aaBB, aaBb	BB, BO
AB	AABB, AaBB, AABb, AaBb	AB

must stand. The statistical basis for this, resting on the observed frequencies of the four groups in various races, and the observed offspring in matings involving group *AB*, has been thoroughly reviewed (Snyder, 1926). The serologic basis for this hypothesis has been discussed in this paper. The genetic formulas of the four groups on both hypotheses are given in table 3.

To conform with the new designations of the groups, the recessive factor, formerly known as *R*, is here designated as *O*. Since the dominant groups *A* and *B* may carry the recessive *O*, it is proper from a genetic standpoint to speak of the groups in terms of their phenotypes, as *O*, *A*, *B*, and *AB*, thus bringing genetic and serologic terminology into line.

The inheritance results to be expected on the two hypotheses are the same except when a parent of group *AB* is involved. A comparison of the two hypotheses in this respect is given in table 4.

The offspring of matings involving a parent of group *AB* constitute critical tests of the merits of the two hypotheses. Crosses of groups $O + AB$ are especially good tests, since the offspring should be only of groups *A* and *B* on the basis of multiple allelomorphs, but of all four groups on the basis of independent pairs of factors. As an example

of the data available for comparing the two hypotheses, table 5 is given, summarizing all of the recorded unions of groups $O + AB$.

As the most striking example of the fundamental discovery that an agglutinogen never appears in a child unless it is present in one of the parents, there are listed in table 6 all of the recorded unions of groups $O + O$. In this mating neither parent possesses any agglutinogen.

TABLE 4.—*Theoretical Results of Blood Group Crosses*

Mating	Independent Pairs	Multiple Allelomorphs
O + O	O	O
O + A	O, A	O, A
A + A	O, A	O, A
O + B	O, B	O, B
B + B	O, B	O, B
A + B	O, A, B, AB	O, A, B, AB
O + AB	O, A, B, AB	A, B
A + AB	O, A, B, AB	A, B, AB
B + AB	O, A, B, AB	A, B, AB
AB + AB	O, A, B, AB	A, B, AB

TABLE 5.—*Recorded Unions of Groups O + AB*

Investigator	Number of Families	Number of Offspring in Group			
		O	A	B	AB
Von Dungern and Hirszfeld, 1910	4	2	2	2	3
Learmonth, 1920	3	0	0	0	12
Avdeieva and Grizevicz, 1921	2	2	0	0	1
Keynes, 1922	1	0	0	2	1
Tebbutt and McConnel, 1922	2	0	7	5	0
Ottenberg, 1922	2	0	5	5	0
Oyanada, 1922	3	0	6	0	0
Buchanan, 1923	4	6	2	2	1
Jervell, 1923	3	0	5	2	0
Dyke and Budge, 1923	1	0	0	1	0
Kirihara, 1924	6	1	9	8	0
Plüss, 1924	7	2	8	8	1
Mino, 1924	4	0	5	4	0
Hirszfeld, Hirszfeld and Brokman, 1924	1	0	2	0	0
Dossena, 1924	7	2	2	3	0
Furuichi, 1925	9	2	7	7	2
Kawaishi and Furuhashi, 1925	4	2	11	8	2
Staquet, J., 1925	2	8	9	2	1
Snyder, 1926	5	0	10	13	0
Furuhata, Ichida and Kishi, 1926	26	1	38	43	0
Total		28	128	115	23
Per cent observed		9.5	43.5	39.4	7.4
Per cent expected, multiple allelomorphs		0.0	50.0	50.0	0.0
Per cent expected, independent pairs		11.1	22.2	22.2	44.4

In both of these tables there are exceptions to rule. In table 5 the exceptions are only to the hypothesis of multiple allelomorphs, and would be in accord with the hypothesis of independent pairs. In table 6, however, the exceptions would not be in accord with either hypothesis. These exceptions, in view of the overwhelming confirmation of most of the results, must be explained either as illegitimacy or on the basis of mistakes in technic. In the light of the recent developments on the questions of titer and pseudo-agglutination in the technic of blood grouping, it is no wonder that exceptions have been recorded.

In the matings of *O* + *AB* (table 6), if it is argued that the finding of children of groups *O* and *AB* by certain investigators is evidence against the hypothesis of multiple allelomorphs, it must be pointed out that the lack of children of groups *O* and *AB* in the results of other investigators is equally evidence against the hypothesis of independent pairs.

Since the statistical treatment of the mass data unconditionally upholds the hypothesis of multiple allelomorphs, and the individual family histories strongly favor it, it must be adopted as the best basis for the inheritance of the groups according to present knowledge.

Medicolegal Application.—On the basis of the older hypothesis, Ottenberg (1921, 1922) presented tables showing how, if the blood group of a child and of one parent were known, it was in some cases

TABLE 6.—*Recorded Unions of Groups O + O*

Investigator	Number of Families	Children in Group O	Children in Other Groups
Von Dungern and Hirszfeld, 1910	11	25	.....
Learmonth, 1920	9	18	1 in group A
Keynes, 1922	2	4	.....
Tebbutt and McConnel, 1922	5	17	.....
Ottenberg, 1922	12	25	.....
Buchanan, 1923	8	17	3 in group AB 2 in group B 8 in group A
Jervell, 1923	2	5	.....
Dyke and Budge, 1923	30	30	.....
Kirihara, 1924	6	20	.....
Plüss, 1924	12	27	.....
Mino, 1924	12	31	5 in group A
Hirszfeld, Hirszfeld and Brokman, 1924	7	19	.....
Dossena, 1924	31	31	.....
Avdeieva and Grizevicz, 1921	16	33	.....
Staquet, 1926	9	35	1 in group A
Snyder, 1926	44	151	.....
Furuhata, Ichida and Kishi, 1926	47	130	.....
Total	268	618	20

possible to determine from this the group of the other parent. The adoption of the newer hypothesis of inheritance changes to some extent the medicolegal applications of the groups. None of Ottenberg's tests are invalidated. On the contrary, the application becomes even more strict. The possibilities of the group of the remaining parent are limited when a known parent of group *AB* is involved.

The medicolegal applications on the newer hypothesis were set forth by Snyder (1927). The table is reproduced here (table 7).

In practice, it will seldom be possible to use the parts of the table involving two or more children. Each child's parentage presents an individual problem, and must usually be considered by itself. The complete list of possibilities is given in the table, however.

As long as any question as to the efficacy of the technic of grouping or the accuracy of the hypothesis of inheritance remains, the use of the medicolegal application must be attended with extreme caution. Two

things are necessary before any character may be used in determining parentage. First, one must be able to recognize without question the presence of the dominant factor in any person. Second, one must be absolutely sure of the hereditary behavior of the character. Until the blood groups can fully meet these requirements, they must be applied in

TABLE 7.—*Prediction of Remaining Parent Groups* *

Known Children in Group	One Parent Known to Be in Group	Other Parent Must Be in Group
O	O	O, A, or B
O	A	O, A, or B
O	B	O, A, or B
O	AB	**
A	O	A or AB
A	B	A or AB
B	O	B or AB
B	A	B or AB
AB	O	†
AB	A	B or AB
AB	B	A or AB
AB	AB	A, B, or AB
O and A	O	A
O and A	A	O, A, or B
O and A	B	A
O and A	AB	**
O and B	O	B
O and B	A	B
O and B	B	O, A, or B
O and B	AB	**
O and AB	O	†
O and AB	A	B
O and AB	B	A
O and AB	AB	**
A and B	O	AB
A and B	A	B or AB
A and B	B	A or AB
A and AB	O	†
A and AB	A	B or AB
A and AB	B	A or AB
A and AB	AB	A, B, or AB
B and AB	O	†
B and AB	A	B or AB
B and AB	B	A or AB
B and AB	AB	A, B, or AB
O, A, and B	O	†
O, A, and B	A	B
O, A, and B	B	A
O, A, and B	AB	**
O, A, and AB	O	†
O, A, and AB	A	B
O, A, and AB	B	A
O, A, and AB	AB	**
O, B, and AB	O	†
O, B, and AB	A	B
O, B, and AB	B	A
O, B, and AB	AB	**
A, B, and AB	O	†
A, B, and AB	A	B or AB
A, B, and AB	B	A or AB
A, B, and AB	AB	A, B, or AB
O, A, B, and AB	O	†
O, A, B, and AB	A	B
O, A, B, and AB	B	A
O, A, B, and AB	AB	**

* In the combinations which are not given in this table, the other parent could be any of the four groups.

** Impossible; neither parent could be of group AB, on the hypothesis of multiple allelomorphs.

† Impossible; neither parent could be of group O, on the hypothesis of multiple allelomorphs.

medicolegal cases sparingly and cautiously. And it must not be thought that the blood group factors have any mysterious potency in such cases by virtue of being hidden in the blood. They have an especially extensive application, it is true, because they are based on three allelomorphs in which the dominant heterozygote is phenotypically recognizable. But color of the eye, direction of whorl in the occipital hairs, or any other pair of characters which are accurately recognizable and whose inheritance is thoroughly understood, may be used in determining parentage.

Lattes (1927) has recently reported a series of cases in which he used the iso-agglutination reaction to identify blood stains. His technic is applicable to very small spots of blood, and even to old stains. Here again, it is a case of eliminating certain persons rather than incriminating any definite one, except as may be done by such elimination.

INFLUENCE OF THE BLOOD GROUPS ON PATHOLOGIC, PHYSIOLOGIC, OR ANATOMIC CONDITIONS DUE TO LINKAGE RELATIONS OR OTHER EFFECTS

Theoretical Considerations.—Once the blood groups are shown to be hereditary, their relations to other human characters become important. Hereditary characters carried on the same pair of chromosomes as the blood groups would be linked with the groups in inheritance. This possibility opens a wide range of speculation from a pathologic standpoint. Hereditary characters carried on other chromosomes would be inherited independently of the blood groups. Nonhereditary factors would be only indirectly influenced by the hereditary nature of the groups.

A considerable number of workers have recently investigated this subject. Many of them concluded that the particular character investigated was linked with the groups in inheritance. All of these conclusions are based on false premises or erroneous ideas of linkage. Snyder (1926 b) pointed out the fallacies in these conclusions and indicated by discussion and tables just what must be shown in any case in order to claim true linkage.

Two things must be borne in mind in any study of linkage of the blood groups: First, linkage can never be demonstrated by means of abnormal distribution of the four groups among persons showing a particular character. Second, owing to the difficulties inherent in the study of human heredity, all family histories involving the simultaneous inheritance of two pairs of characters will not give data on linkage. In animals, in which controlled matings make possible the determination of the genetic formula of any individual, and in which large numbers of offspring may be raised from any type of cross, studies of linkage are comparatively easy. But in man, the genetic composition is hard to determine, and controlled matings are not available; therefore, the problem becomes more difficult. It is almost impossible to determine

in certain families whether the coupling or the repulsion phase is involved, and therefore which of the offsprings are cross-overs and which are non-cross-overs. It is likewise practically impossible to be sure that a dominant parent is homozygous, or, as is more to the point, to know whether a dominant parent is heterozygous in the absence of recessive offspring.

As already pointed out (Snyder, 1926 b), there are four possibilities in considering the relations of the blood groups to other characters:

1. The possibility of complete linkage of some hereditary factor with one of the blood group factors. In this case, if the mutation causing the character had taken place but once, the character, if recessive, would be confined to group *O*, or to group *A*, or to group *B*, and would not appear in any of the others. If dominant, the character might also appear in group *AB*. For example, if a recessive mutation causing feeblemindedness had taken place on the chromosome carrying the blood group factor *A*, all feebleminded persons would be group *A*. As a matter of fact, however, feebleminded persons occur in all four groups. Even so, there might still be complete linkage if the mutation had taken place several times, once on a chromosome carrying *A*, once on a chromosome carrying *B*, and once on a chromosome carrying *O*. In such a case, inheritance studies would quickly reveal the fact of complete linkage. Complete linkage is hardly to be expected, however, and the next possibility is of much more importance.

2. The possibility of partial linkage of some hereditary factor to the blood group factors. In this case, although the original mutation would have been linked with but one of the blood group factors, subsequent crossing over would in time distribute the factor proportionately among the four groups. Therefore, linkage cannot be detected by observing the percentages of the four groups among various anatomic or pathologic conditions. This has not been appreciated by many investigators.

When partial linkage occurs, it may be demonstrated only by careful pedigrees for at least two generations. When these are traced, the data will in some cases lend themselves to analysis from the standpoint of linkage. All family histories will not furnish data which can be used, however.

When only two generations are studied, unless all dominant parents show some recessive children, no data on linkage is of value, since a single human family is never large enough to remove all possibility of the parents being heterozygous when they give all dominant children. For example, if susceptibility to a certain disease is recessive to non-susceptibility, all families must show at least one susceptible child before being of value as linkage data. In the case of the blood groups themselves, all matings must show at least one child of group *O*, except

when one parent is of group *AB*, in which case the matings must show at least one child of each of groups *A* and *B*.

When three generations are studied, it may be possible in some cases to show that a dominant parent is heterozygous because one grandparent was dominant, the other recessive. In any case, sufficient combinations must appear in the offspring to give some basis for deciding between linkage and independent assortment. The minimum number of combinations will vary with the mating.

3. The possibility of independent inheritance of the blood group factors and the factor under discussion. This is most often to be expected, because of the large number of chromosomes in man, and will give all possible combinations in inheritance, in typical dihybrid ratios.

4. The possibility that one or another of the blood group factors may in addition tend to affect indirectly some anatomic, physiologic or pathologic condition, thus causing an abnormal distribution of the groups in the particular condition affected. Although not probable, this is a possibility which must be considered. In such a case, the condition affected, if hereditary, would be limited by the blood groups, and would give abnormal hereditary behavior. If not hereditary per se, the condition would become imperfectly hereditary by reason of its partial dependence on the blood groups. As a hypothetical example, one may assume that the blood group factor *A* also affects the red cells in such a way that they more readily resist the entrance of the malarial parasite. Then groups *A* and *AB* would not be so well represented among malarial patients, and groups *O* and *B* would be represented in greater proportion than they are found among the general population. The finding of this abnormal distribution of the groups in a pathologic condition would not be a basis for concluding that the condition was an inherited character linked with the groups, however. On the contrary, as has already been pointed out, if there were true linkage, the distribution of the groups would be normal.

A fifth possibility, much restricted and only hypothetical, may be added here. If Bernstein's hypothesis of the essential similarity of all serums as to iso-agglutinins is correct, there might well be disarrangements of some sort in children at the onset of the agglutinin. In this case the effect of the reciprocal binding would be most marked in children of group *AB*, and would not occur at all in children of group *O*. This phenomenon, if it occurs, must be restricted to sudden, apparently spontaneous disarrangements in children of groups, *A*, *B* and *AB*, from birth to about 2 years.

Practical Observations.—Before any abnormal distribution of blood groups can be accepted as certain, large numbers of cases must be studied. The small numbers usually reported may easily be chance variations. Alexander (1921), in a study of fifty cases of malignant

disease, found that group *O* was not so well represented as among the general population, while groups *B* and *AB* were better represented. Buchanan and Higley (1921) and Pfahler and Widmann (1922), however, found all groups represented in proportions similar to those found in healthy people. Johannsen (1925) stated that group *O* is represented less than in normal persons, but the discrepancy is slight. Among eighty-four cases of carcinoma, Weitzner (1925) found considerably less group *O* blood than normal and considerably more group *AB* blood. Schiff (1926), however, takes exception to these results, stating that patients with cancer show the same proportions of the blood groups as healthy people from the same locality. He considers Weitzner's results due to error from pseudo-agglutination, which is frequent in the blood of patients with cancer. Hoche and Moritsch (1926) found an abnormally large percentage of group *AB* among 176 cases of cancer. Bendien (1926) found in 110 patients with cancer a low percentage of group *O* and a high percentage of group *AB*. Hirszfeld and Hittmair (1926), on the other hand, found a normal distribution among 150 cases of cancer. Until the possibility of pseudo-agglutination is ruled out, the abnormal distributions cannot be accepted as proved. It will be noted that the reported increase is always in the direction of more agglutination (group *AB*) away from the lack of agglutination (group *O*), a fact which, coupled with the frequency of pseudo-agglutination in the blood of patients with cancer, makes controls such as mechanical agitation, lecithin or kaolin absolutely necessary.

In this same connection, both Straszynski (1925) and Wiechmann and Paal (1926) found group *O* poorly represented among cases in which the Wassermann reaction was positive, while group *AB* was over-represented. In addition, Straszynski found that a larger percentage of group *O* patients recovered under treatment.

Cathala and LeRasle (1925) concluded that incompatibility of maternal and fetal blood is not responsible for eclampsia or other complications in pregnancy. Ohnesorge (1925) found that incompatibility of mother's and child's groups has no connection with toxicoses of pregnancy. Schneider (1925), however, stated that toxemia does not accompany pregnancy when groups of mother and of child coincide. He considers that a divergence in blood groups causes a disposition toward eclampsia. Allen (1926) found that the percentages of incompatibility between the blood of the mother and that of the infant were similar in normal women and in cases of late toxemias of pregnancy. He concluded that there was no evidence that the late toxemias of pregnancy have their origin in the iso-agglutination phenomena.

Kaczynsky (1926) found that the percentage of persons susceptible to the Dick test is approximately the same in each of the four groups.

The same holds true of the Schick test. Holló and Lénard (1926) and Alperin (1926) found a normal distribution of the groups among patients with tuberculosis. Mironescu and Stefanoy (1926) gave figures on the percentages of the blood groups among persons with various diseases, but the numbers are entirely too small to be of much value. Chi-pan (1924) found a normal distribution in cases of tuberculosis, syphilis and typhoid fever.

Feldmann and Elmanowitch (1925) found a high percentage of group *AB* in 815 cases of mental diseases. In paralytic patients the occurrence of *AB* rose as high as 33 per cent. These authors consider the blood group as aids in psychiatric diagnosis.

Gundel (1926 a) reported an increased percentage of group *B* in a series of 402 cases of mental diseases. Snyder (1926), however, found normal distributions in several hundred cases of epilepsy, dementia praecox and feeblemindedness. Jacobsohn (1926) found the distribution normal among patients with general paralysis. Gundel (1926 b) reported a normal distribution among criminals. Böhmer (1927), however, found an increased percentage of *B* (20 per cent) among 1,034 criminals. Wilczkowski (1927) found an excess of group *AB* among patients with paresis, but a normal distribution among patients with schizophrenia. Bunker and Meyers (1927) reported that the groups were normally distributed among a series of patients with general paralysis.

Percentages of the blood groups among persons of the same race differing in pigmentation, in color of hair, in form of head and in body build have been recorded, but without showing any significant differences (Hirszfeld and Hirszfeld, 1919; Dossena, 1924, 1925; Klein and Osthoff, 1926).

Hirszfeld and Zborowski (1926) consider that considerable differences in the blood groups of husband and wife may retard the developing embryo, and extreme differences may even affect fecundity. They go so far as to suggest certain groups which may be sterile when mated together. At least part of their conclusions are rendered invalid when their material is considered from the standpoint of the newer hypothesis of inheritance.

In studies of linkage of the blood groups, family histories have been given only by Hirszfeld, Hirszfeld and Brokman (1924), Fürst (1925), Levine (1926) and Snyder (1926). Hirszfeld, Hirszfeld and Brokman present family histories of the simultaneous inheritance of the blood groups and susceptibility to the Schick test. They conclude that susceptibility to diphtheria is linked with the blood groups. On this basis, Hoche and Moritsch (1925) have assumed that from a knowledge of the blood groups of a family and the Schick reactions of the parents, accurate knowledge of the susceptibility of the children can be obtained. It was pointed out (Snyder, 1926 b) that Hirszfeld's family histories

do not indicate linkage any more than free assortment, and that the attempt to draw conclusions based on the assumption of linkage is unwarranted and dangerous. Even if there were linkage, any appreciable amount of crossing over would obviate the possibility of making any practical clinical applications.

It is conceivable that although no linkage is concerned, the blood group factors might indirectly affect susceptibility to diphtheria, in a manner such as that outlined above in possibility 4. In this case, however, an abnormal distribution of the groups among susceptible patients would be expected, a condition which apparently does not occur.

Fürst (1925), on the basis of four family histories, concluded that susceptibility to goiter was linked with the blood groups. It was pointed out that there was no evidence for linkage in Fürst's data.

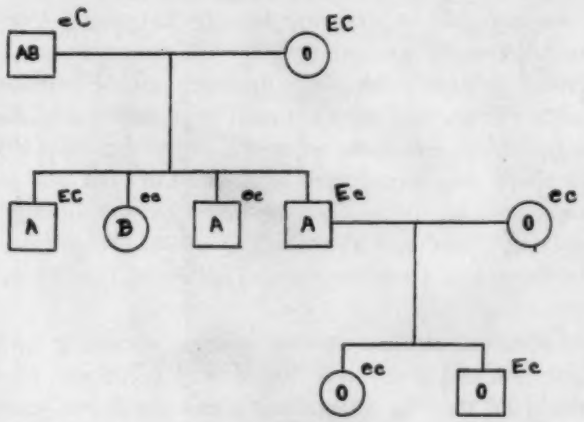

Fig. 2.—Family history showing the simultaneous inheritance of eye color, direction of hair whorl and the blood groups. *E* indicates brown eyes; *e*, blue eyes; *C*, clockwise; *c*, counter-clockwise.

Levine (1926) found that atopic hypersensitiveness was not linked with the blood groups. Snyder (1926 b) stated that color of eyes did not seem to be linked with the groups. The question of linkage with various inherited characters is being investigated in my laboratory at present. A pedigree involving the simultaneous inheritance of blood groups, color of eyes and direction of whorl in the occipital hairs is given in figure 2. This shows crossing over between the blood groups and color of eyes, and between the blood groups and the direction of hair whorl, and indicates that these characters are inherited independently of the groups. As more human hereditary characters become available, their linkage relations to the blood groups and to each other should be carefully studied.

At present it has not been proved that any character is linked with the blood groups in inheritance, and no character has as yet been

unquestionably found to be indirectly influenced by the groups. Contrary conclusions are based in every case on insufficient or irrelevant data. The extension and medical application of such erroneous conclusions may not only be confusing and valueless, but they may be dangerous to life.

SEROLOGIC CLASSIFICATION OF RACES

Blood Groups in Animals.—The question of the occurrence of blood groups in the lower animals has never been satisfactorily settled. Most investigators have failed to find fixed groupings in animals below the anthropoid apes. A few workers, however, have reported the finding of iso-agglutinins and iso-agglutinogens, on the basis of which groups have been formed.

Hektoen (1907) studied rabbits, guinea-pigs, dogs, horses and cattle, but was not able to find any iso-agglutination. Von Dungern (1910) concluded from experiments by the adsorption method that there were four groups in dogs. Ottenberg and Friedman (1911), in studies with rabbits and steers, found that they could demonstrate four groups in rabbits and three in steers. Ingebrigsten (1912) found that in cats there was occasional agglutination, but no satisfactory grouping could be made. He likewise failed to find iso-agglutinins in dogs. Ottenberg, Friedman and Kalisky (1913) confirmed the lack of groups in dogs, and Ottenberg and Thalhimer (1915) did likewise for cats.

In 1913, Fishbein studied several species, including swine, cattle, sheep, rabbits, cats and dogs. He found only occasional iso-agglutination, and concluded that the phenomenon was much less pronounced in animals than in man, and permitted of no satisfactory grouping. Weszeczky (1920) found no iso-agglutination in cattle, rabbits, guinea-pigs and chickens. In dogs, horses and swine he found occasional iso-agglutination, but was unable to make any groupings. Rhodenberg (1920) failed to find agglutination in various strains of rats. Likewise MacDowell and Hubbard (1922) did not observe agglutination in 1,180 combinations of serum and cells of diverse strains of mice.

Pannisset and Verge (1922) could not detect any evidence of fixed blood groups in either horses or cattle. Przesmycki (1923) found no evidence of iso-agglutination in rabbits. Hirszfeld and Przesmycki (1923), however, distinguished three groups in horses similar to the iso-agglutination groups in man, but less regular. Landsteiner and van der Scheer (1924) found a similar grouping. Walsh (1924) found irregular iso-agglutination in horses, but could not demonstrate any grouping. Bialosuknia and Kaczkowski (1924) reported the occurrence of three groups in sheep. Snyder (1924), using practically every known variety of the domestic rabbit, was unable to find any iso-agglutination.

Von Dungern and Hirszfeld (1911) studied two sets of chimpanzee serum and one set of erythrocytes. The bloods corresponded to the human group A. The cells of macaque monkeys could not be grouped with human serums.

Landsteiner and Miller (1925) examined the blood of twenty-one anthropoid apes, and seventy-six lower monkeys of thirty-six species. The erythrocytes were grouped by means of human serums or adsorbed immune rabbit serums. Since hetero-agglutinins for anthropoid corpuscles are present in human serums, the investigators had first to obviate the effects of these, which they did by adsorbing the iso-agglutinins and then freeing them again by themselves, or by immunizing rabbits. Of the twenty-one apes studied, eighteen possessed iso-agglutinogens, which were found to be identical with those of human

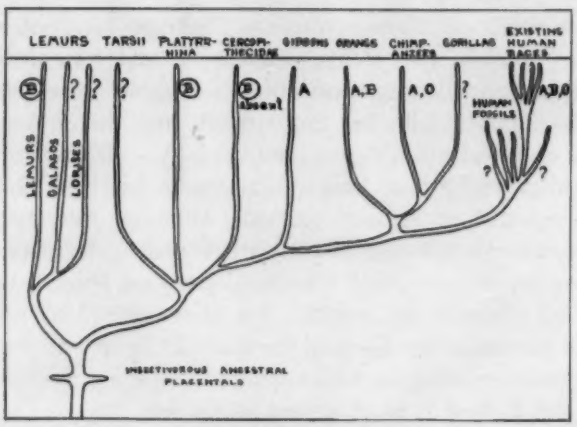

Fig. 3.—The occurrence of agglutinogens among the anthropoid apes and the lower monkeys. *A, B,* iso-agglutinogens identical with human iso-agglutinogens. Encircled *B,* iso-agglutinogen similar to human iso-agglutinogen *B,* but not identical with it. (After Landsteiner and Miller.)

blood. Of the fourteen chimpanzees studied, eleven belonged to group *A* and three to group *O*. Of the six orang-utans, two belonged to group *A*, three to group *B* and one to group *AB*. The one gibbon studied belonged to group *A*. In the lower monkeys, only a factor similar to human iso-agglutinogen *B*, but not identical with it, was found. In figure 3 is given the schematic representation of the iso-agglutinogens now known to be found in the primates. Further investigation may extend this list, since only small numbers have been studied thus far.

As a general rule, blood of one species of animals contains hetero-agglutinins for cells of other species. Interesting exceptions to this rule have been noted. Weszeczky (1920) found that the serum of the buffalo did not agglutinate the cells of cattle. MacDowell and Hubbard (1922) recorded that mouse cells were not agglutinated by rat serum.

Walsh (1924) found that horse serum did not agglutinate ass cells, although in most cases ass serum agglutinated horse cells. Mule serum did not agglutinate either horse or ass cells, nor were mule cells agglutinated by horse or ass serum. Landsteiner and van der Scheer (1924) obtained similar results, but found that certain mule serums agglutinated certain horse bloods, while some mule cells were agglutinated by certain horse or ass serums.

Lack of agglutination has been assumed by the absence of reactions after transfusion in certain cases. Friedenthall (1900) found no ill effects in a cat after transfusion of ocelot blood into it. There were also no ill effects when transfusion was effected between rabbit and hare, or between rat and mouse.

The possible taxonomic importance of such results as these is being investigated in my laboratory.

Blood Groups in Human Races.—The demonstration in 1919 by L. and H. Hirszfeld that the percentages of the four groups vary in different races, and that this variation is roughly correlated with geographic distribution, laid the groundwork for the present extensive application of blood grouping to anthropology. Because of the nature of the blood grouping tests, much of the work has been done by physicians and reported in medical journals, although recently anthropologists and geneticists have entered the active lists. The Hirszfelds' work was a powerful stimulus, and investigations were immediately made on peoples in all parts of the world. Yet Hirszfeld (1927) records that a reputable journal, after keeping the manuscript nine months, returned it with the comment that the subject matter would not interest physicians. The work finally had to be published in English.

The Hirszfelds took advantage of the concentration of races on the Macedonian battlefront during the World War, and grouped the bloods of 500 members of each of sixteen different peoples. A summary of their results showed that factor *A* decreased from west to east, while factor *B* increased. The sixteen peoples could be roughly divided into three types, based on the relative amounts of *A* and *B*. As a serologic criterion the Hirszfelds suggested a "biochemical index," to be arrived at by dividing the percentage of *A* in a given race by the percentage of *B*, that is, by dividing the percentage of groups $A + AB$ by the percentage of groups $B + AB$. Of the Hirszfelds' three types, the European type had an index of 2.5 — 4.5 (more than twice as much *A* as *B*), the intermediate type had an index of 1.3 — 1.8, and the Asio-African type varied from 0.5 to 1.09. Later reports from many investigators have given indexes of intermediate magnitudes, so that no sharp divisions exist at present on this basis.

It was pointed out by Snyder and by Bernstein in 1925 that the biochemical index was not mathematically adequate, and must be discarded. In its place must be used the only correct criteria from a

statistical standpoint, the frequencies of the hereditary factors concerned. These may be arrived at as follows: Let p equal the frequency of the factor A, q equal the frequency of the factor B, and r equal the frequency of the factor O in any race. Then $p+q+r=1$. The possible combinations of these factors are expressed as $(p+q+r)^2$. In this way the following combinations are formed:

	A p	B q	O r
A p	AA p^2	AB pq	AO pr
B q	AB pq	BB q^2	BO qr
O r	AO pr	BO qr	OO r^2

From this the proportions of the four groups are reckoned as follows:

$$\text{group } O = r^2$$
$$\text{group } A = p^2 + 2pr$$
$$\text{group } B = q^2 + 2qr$$
$$\text{group } AB = 2pq$$

From this p, q, and r are calculated.

$$\text{Group } O + \text{group } A = r^2 + 2pr + p^2 = (r+p)^2$$
$$r + p = \sqrt{\text{group } O + \text{group } A}$$
$$p = \sqrt{\text{group } O + \text{group } A} - r$$
$$p = \sqrt{\text{group } O + \text{group } A} - (1 - p - q)$$
$$q = 1 - \sqrt{\text{group } O + \text{group } A}$$

Similarly,

$$p = 1 - \sqrt{\text{group } O + \text{group } B}$$
$$r = \sqrt{\text{group } O}$$

On this basis, p, q, and r can be calculated for any people in which the percentages of the four groups are known. It is obvious that the more persons studied, the more accurate the frequencies will be. Also, within mixed races, the individual components must be studied separately as far as possible.

Ottenberg (1925), perceiving that the biochemical index was not entirely adequate, formulated a classification of peoples based on the

percentages of the groups. He recognized six types. A more accurate result is attained, however, by basing the classifications directly on the frequencies p, q and r. Classifications on this basis have been offered by Snyder (1926) and Streng (1926). The graphic figure presented by Streng is theoretically in three dimensions, but is presented as a plane due to the limitations of flat printing. Three axes, *PP*, *QQ* and *RR* are used, corresponding to the frequencies of the inherited factors, and the position of each people on these axes is plotted from

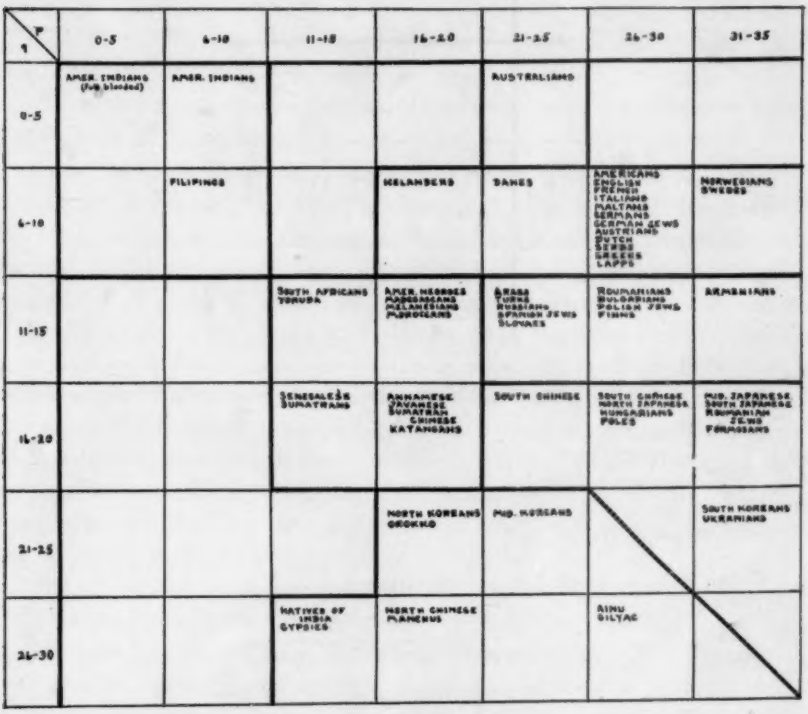

Fig. 4.—Correlation of p and q among various peoples of the world.

the frequencies of the three factors in that people. The various races studied fall into natural groups on the figure.

A more easily understood chart based on the frequencies of the inherited factors was presented by Snyder (1926). In this figure the frequencies p and q were grouped on a correlation table; the frequency r was ignored, as two races having similar frequencies of p and q would have similar frequencies of r, since $p + q + r = 1$. If desired, values of r could be added to this chart as a third dimension. The figure is reproduced here, with the addition of new peoples which have been studied since the first appearance of the chart (fig. 4).

The investigators responsible for the study of each people in the original figure are fully listed in Snyder (1926). Workers responsible for the new peoples in the present chart are as follows: Lapps, unpublished data from this laboratory, also Schött (1926) and Rietz (1927); Belgians, Staquet (1926); Moroccans, unpublished data from this laboratory; Katangans, Bruynoghe and Walravens (1926); Yoruba tribe of the West Africans, Muller (1927); Finns, unpublished data from this laboratory, also Streng (1926); Formosans, Furuhata and Kishi (1926); Gilyac, Kishi (1925, 1926); Orokko, Kishi (1925, 1926).

It will be seen that the various peoples fall more or less into natural groups. A certain empiric manner of grouping must be admitted, since p and q have been lumped into 5 per cent classes. However, each square in the figure contains nationalities which agree in their frequencies p, q and r. Snyder emphasized the fact, however, that it is not implied that because two peoples occur in the same portion of the figure, they have the same racial history, but only that they contain similar amounts of A and B. For convenience, the various peoples were arbitrarily divided into types, set off by heavy lines on the figure. These types agree to a certain extent with Ottenberg's (1925). The types recognized will be briefly outlined, and the details of the included peoples may be obtained from the figure.

1. The European type, consisting of all West-European peoples, and some others, and containing a high frequency of A and a low frequency of B.

2. The intermediate type, containing a decreased frequency of A and an increased amount of B, and consisting of peoples such as the Arabs, Turks and Roumanians, lying geographically intermediate between Europe, with its high frequency of A, and Asia, with its high frequency of B.

3. The Hunan type, with an exceptionally high frequency of A combined with a moderate B-frequency. In it are included South Chinese, South Koreans and Japanese and in addition Hungarians and Poles from Europe.

4. The Indomanchurian type, showing a high B-frequency and a low amount of A. A striking feature brought out here is the inclusion of North Chinese, North Koreans and Manchus, in contrast to the South Chinese, South Koreans and Japanese included in the preceding type. Ainu, Gilyac, gypsies and natives of India also find a place here.

5. The Africo-Malaysian type, with both agglutinogens moderately developed, and including all the Africans and Malays as yet studied.

6. The Pacific-American type, in which neither agglutinogen is well developed. American Indians and Filipinos are included here.

7. The Australian type, distinct because of the high frequency of *A* with almost a total absence of *B*.

These types, as has been stated, are purely arbitrary, and are subject to constant revision. They merely serve as a working basis for racial classification on serologic grounds. The world distribution of the seven types is shown in figure 5.

It must be emphasized that the blood group factors do not constitute a more potent source of classification than any other hereditary factors with this exception: there is no possibility of a conscious selection in the case of the blood groups. Any selective value the blood groups may

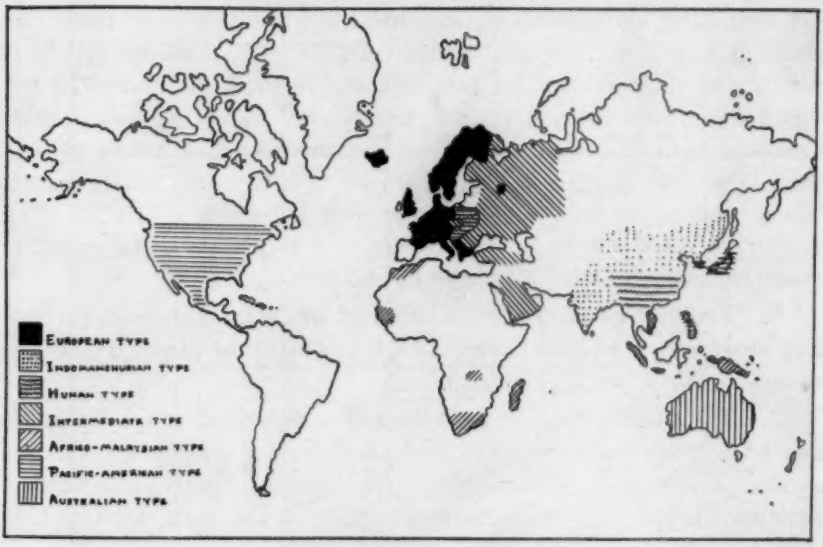

Fig. 5.—Distribution of the seven types throughout the world.

have (and none has been shown as yet) would be due to a secondary relation with some other factor that did possess a selective value of its own. But it must not be thought that because the groups are hidden in the blood they possess some mysterious power in providing a basis for racial classification. They must merely take their place as available factors along with pigmentation, hair form, cephalic index and the rest.

Because of the hereditary nature of the groups and their stability under rather extreme environmental influences (such as drugs, anesthesia, roentgen-ray irradiation) it is to be expected that the proportions of the groups would remain constant from generation to generation in any race in the absence of crossing, and that when crossing did occur, a definite change in the proportions of the groups would be brought about.

The first of these expected results has been demonstrated by Verzár and Weszeczky (1922). They found that gypsies, a people from India living in Hungary for several hundred years, showed the same proportions of the groups as the natives of India studied by Hirszfeld and Hirszfeld, quite different from those of the Hungarians surrounding them. The actual figures are given in table 8.

The second of these expectations, namely, that racial crossing should modify the proportions of the groups, was demonstrated by Snyder (1926). In a study of American Indians, who have small amounts of *A* and *B* bloods, it was possible to work out a graded series from pure-blooded Indians to whites, showing a constant increase in the agglutinogens. These results are given in table 9.

TABLE 8.—*Illustrating the Constant Percentages of the Groups in the Absence of Crossing*

Race	Number Studied	Per Cent in Group				p	q	r
		O	A	B	AB			
Natives of India	1,000	31.3	19.0	41.2	8.5	14.9	29.1	55.9
Gypsies (in Hungary)	385	34.2	21.1	38.9	5.8	14.5	25.6	58.4
Hungarians	1,500	31.0	38.0	18.8	12.2	29.4	17.0	55.6

TABLE 9.—*Graded Series Showing the Effect of Crossing on the Proportions of the Groups*

Race	Number Studied	Per Cent in Group			
		O	A	B	AB
Indians said to be pure	453	91.3	7.7	1.0	0.0
All Indians (mixed and pure)	1,134	79.1	16.4	3.4	0.9
Indians known to be mixed	409	64.8	25.6	7.1	2.4
Americans (white)	1,000	45.0	42.0	10.0	3.0

The Indians said to be pure showed such low percentages of *A* and *B* that it was thought that true full-blooded Indians would be all group *O*. Even those said to be pure may have had some white admixture, as the records went back only three or four generations. It has also been considered by most authors (Schiff and Adelsberger, 1924; Bernstein, 1925; Staquet, 1925; Snyder, 1926) that the human race was originally all of group *O*, the factors *A* and *B* having arisen separately by later mutation. Migration and intermingling have been considered as the agencies of spread of the factors. On this basis it is postulated that certain races, such as the American Indians and Filipinos, became isolated from the rest of mankind before mutations *A* or *B* took place, thus accounting for the low frequencies of these factors in these races. Further evidence for this view is found in the fact that all the peoples showing more than 50 per cent of group *O* are island folk or otherwise isolated. Mutation *A* is assumed to have occurred in Europe

and mutation *B* in India or the Orient, from which they were carried and intermingled by migrations. Migration charts and maps have been constructed on the basis of the biochemical index by Steffan (1925) and others.

This view of the situation, however, has received a severe blow by the work of Landsteiner and Miller, who demonstrated the factors *A* and *B* among anthropoid apes. If these factors are identical with the corresponding factors of human beings (and tests so far indicate that they are), the origin of the mutations must probably be placed before the origin of present-day man. This opens up an entirely new range of possibilities concerning the origin and distribution of the groups.

Various workers have at times challenged the validity of group percentages in any race as anthropologically significant (Schütz and Wohlisch, 1924; Lanner, 1925; Grove, (1926). These objections have been based on the observations that the range of variation within a race may be as great as the variations between different races. These objections do not seem warranted, however. In the case of the Germans, the numbers studied in the various regions are too small to allow statistical significance to the differences. In the case of the Ainu, the various regions are isolated (islands), and inbreeding of small original stocks may well have carried on originally insignificant differences.

The blood groups, then, dependent on fixed unit hereditary factors, take their place along with other hereditary characters as aids in racial classification. At present their application is limited by the fact that their distribution is known among many nationalities, but among few races. When they are carefully studied from a true racial standpoint, their phylogenetic origin worked out and their possible selective value understood, the extent of their usefulness as aids to racial classification will become apparent.

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Notes and News

University News, Promotions, Resignations and Appointments.—At the Mayo Foundation, Dale L. Martin has been appointed fellow in pathology, and Carlos Gustavino, fellow in surgical pathology.

Virgil H. Moon, head of the department of pathology in the Indiana University School of Medicine, has been appointed head of the department of pathology at Jefferson Medical College, Philadelphia, and will assume his duties next autumn.

Peritz M. Kurzweil has been appointed assistant professor of pathology in the New York Post-Graduate Medical School and Hospital.

R. G. Hussey has been promoted to professor of pathology in Yale University.

A fellowship in the chemotherapy of cancer will be established at Cornell University Medical College under the terms of a gift of $5,000 a year for five years, made by L. N. Littauer, of Gloversville, according to an announcement of Dr. James Ewing, professor of pathology in the college.

Bela Halpert, instructor in anatomy at Johns Hopkins University, has been appointed assistant professor of pathology in the University of Chicago.

Kaethe W. Dewey has resigned her position as assistant professor of oral surgery (pathology) in the University of Illinois, and has accepted a similar position in the college of dentistry of the University of Pittsburgh.

Arthur I. Kendall, formerly professor of bacteriology and dean at Northwestern University Medical School, has resigned as professor of bacteriology and public health at Washington University School of Medicine, St. Louis, to accept reappointment as professor of bacteriology at Northwestern University.

Hamilton R. Fishback, pathologist at Wesley Memorial Hospital and associate in pathology in the Northwestern University Medical School, has been promoted to assistant professor of pathology and will devote his whole time to work at the medical school.

Death of Karl Ipsen.—Karl Ipsen, professor of forensic medicine in the University of Innsbruck, has died at the age of 72.

Edward N. Gibbs Memorial Prize Fund.—The income of this fund, about $1,000 annually for three years, is available for research in problems of renal disease. Applications for grants may be made to Committee of Edward N. Gibbs Memorial Prize Fund, New York Academy of Medicine, New York.

Death of Abraham Zingher.—Dr. Abraham Zingher died in the laboratory of the Willard Parker Hospital from asphyxia, owing to the accidental leakage of gas. He was born in Roumania in 1885, and received his medical degree from

Cornell University. He was assistant director of the bureau of laboratories of the Health Department of New York, and assistant professor of bacteriology in New York University. Probably his best known work was the immunization of children against diphtheria.

Awards of John Scott Prizes.—John Scott bronze medals and $100 awards have been given to Alfred S. Hess for his work on the influence of ultraviolet light on rickets and on the vitamin value of food; to Peyton Rouse for his work on sarcoma in fowls caused by a filtrable agent, and to Afranio Do Adaral for work on serum against snake venom. In 1816, John Scott of Philadelphia established a fund for awards "for useful inventions for the use and benefit of mankind."

Medical Examiner Act Passed in New Jersey.—This state has enacted a law that makes it possible for first class counties to adopt a medical examiner system under which medical examinations are taken out of the hands of the coroner. The New Jersey law is practically the same as the medical examiners' act in New York. Essex County, N. J., has adopted the new law, and Harrison S. Martland of Newark has been made chief medical examiner. This is another step in doing away with the antiquated and cumbersome coroner's office.

Necropsies in Chicago Hospitals.—In 1925, in thirty-nine hospitals in Chicago, each with fifty or more beds, there were 9,519 deaths, and 1,604 necropsies by permission. The total number of necropsies is somewhat smaller than in 1924 and 1923 but twice as large as in 1919. The percentages of necropsies by permission in the number of deaths in the reporting hospitals in 1925 varied from 0.7 to 56.1; in twenty-two hospitals the percentages were higher than 10; in fifteen, higher than 30; in nine, higher than 30, and in six, higher than 40.

Award of Trudeau Medal.—The National Tuberculosis Association, at its recent meeting in Indianapolis, awarded the Trudeau medal to Dr. Edward R. Baldwin of Saranac Lake for his work in the campaign against tuberculosis.

Abstracts from Current Literature

Pathologic Physiology

RESPIRATORY EXCHANGE OF THE FETUS. H. J. STANDER, Am. J. Obst. & Gynec. **13**:39, 1927.

The respiratory quotient was determined by dividing the difference in carbon dioxide of the venous and arterial blood by the difference in oxygen of the venous and arterial blood. The blood was collected from a segment of the umbilical cord before the child began to breathe, and separate analyses were made of the venous and arterial bloods. The oxygen and carbon dioxide were expressed in per cent by volume. Stander concludes that the human fetus has a respiratory quotient of approximate unity. Ether anesthesia and asphyxia affect the respiratory quotient of the fetus, the former demonstrably raising it. From the study of the respiratory quotient of the child, it appears that it utilizes carbohydrates as a source of its energy.

A. J. KOBAK.

THE SURVIVAL VALUE OF THE ADRENAL CORTEX. R. L. ZWEMER, Am. J. Physiol. **79**:641, 1927.

Experimenting on cats, the writer found that following double extirpation of the suprarenals, death ensued in from one to five days, the average being fifty-three hours. Unilateral extirpation had no ill effect. On leaving the cortical portion of one gland intact, with undisturbed blood supply, the animals survived indefinitely, although death followed the removal of this remnant in from one to eight days. The fact that the removal of the medulla was complete was checked by microscopic examination. One animal was observed in which an accessory gland of cortical material preserved life after suprarenalectomy. Transplants of cortical tissue preserved life for about six days, but death eventually followed degeneration of the graft.

H. E. EGGERS.

THE PROLONGATION OF LIFE AFTER EPINEPHRECTOMY. R. L. ZWEMER, Am. J. Physiol. **79**:658, 1927.

On removal of the thyroid glands of epinephrectomized cats, the survival period was lengthened to an average of 200 hours. The excessive elimination of water, which characteristically follows complete extirpation of the suprarenals, was apparently decreased, and the symptoms of suprarenal insufficiency, which are of the character of those of anhydremia, were postponed. With animals fed thyroid substance in the interval between the removal of the two suprarenals, elimination of water was increased, and there was an average survival period of only eighteen hours following removal of the second suprarenal. A 5 per cent dextrose solution, given by mouth in sufficient quantity, prolonged the survival period of epinephrectomized cats to an average of 220 hours.

H. E. EGGERS.

THE BLOOD CHANGES FOLLOWING BILATERAL EPINEPHRECTOMY IN CATS. W. W. SWINGLE, Am. J. Physiol. **79**:666, 1927.

The blood changes observed may be summarized as follows: marked increase of phosphorus, protein, nonprotein nitrogen and urea; marked reduction of

blood sugar and concentration of blood, accounting for the increase in phosphorus and total nitrogen.

A diminished urinary output, with the appearance of albumin in the urine, and with slight pathologic changes in the kidneys, small areas of cortical hemorrhage and cloudy swelling of the renal tubules, was also observed.

It is suggested that death in this condition may be due either to hypoglycemia or to acid intoxication. When the hypoglycemia is corrected by the administration of dextrose, death eventually occurs with symptoms of acid intoxication. It is believed that this is due to inability of the kidney to eliminate acids.

H. E. Eggers.

The Acid-Base Equilibrium of Epinephrectomized Cats. W. W. Swingle and A. J. Eisenman, Am. J. Physiol. **79**:679, 1927.

The heart blood of cats was analyzed for the various acid ions, p_H, and total base content, in the interval between the successive removal of the suprarenals, and was compared with similar data after total removal, with the following results:

There was a fall in p_H, carbon dioxide tension, serum bicarbonate, and total acid, indicating an uncompensated nonvolatile acidosis.

This acidosis appeared to be the result of increased phosphoric and organic acids. An increase of blood protein was also observed, which suggested dehydration.

H. E. Eggers.

Renal Dwarfism. F. W. Lathrop, Arch. Int. Med. **38**:612, 1926.

A case of severe renal insufficiency in association with a curious failure of the development of the bones is reported. Phosphate retention with resulting low blood calcium over a long period may be the cause of delayed ossification of the bones. The severe acidosis, which had been present, may have been the factor which caused the ionization of a sufficiently large proportion of the calcium to prevent the onset of tetany.

S. A. Levinson.

Capillary Permeability and the Inflammatory Index of the Skin in the Normal Person as Determined by the Blister. W. F. Petersen and D. A. Willis, Arch. Int. Med. **38**:663, 1926.

A group of sixty-six normal students were examined with the following procedure: A small cantharides plaster was placed on the arm; it was removed in about six hours, and the time when blister formed was noted. The exudate and blood serum were examined for proteins. This is a relatively simple method of obtaining information concerning the constitutional reactivity of the person. In the normal students the permeability ratio varied from 57 to 84, the inflammatory index from 3.5 to 18.5. Averages were 68 and 10.5, respectively. The permeability is determined by comparing the amount of protein in the blister to the serum protein. The degree of inflammatory reactivity (inflammatory index) is expressed by the quotient $\frac{\text{permeability ratio}}{\text{blister time}}$. In the relatively impermeable group were university athletes and sensitized persons who had some evidence of kidney disturbance. Examined on the basis of inflammatory reactivity, the students who had more than the average amount of parenchymal tuberculosis gave low results (sympathetic), as did also the students with kidney disturbance (sympathetic tonus of arterioles, increased permeability of capillaries). There is definite relation between the blood

pressure and permeability. There is some evidence of relationship between influenza morbidity and permeability (increased permeability following influenza and scarlet fever [?]). Students in the impermeable group respond to the subcutaneous injection of epinephrine hydrochloride with less systemic vascular effect (greater local effect at site of injection). There is a direct chemical basis for the differences in permeability and inflammatory response, namely, the ratio of calcium and potassium. A low inflammatory index is associated with a high blood potassium-calcium ratio, a high inflammatory index with a low potassium-calcium ratio. In the tissue cells these conditions will be reversed. The impermeable cell has in general a greater amount of calcium in proportion to the potassium, while the permeable cell has a small amount of calcium in proportion to the potassium. In infants the permeability of the skin capillaries is probably not high, but in children the permeability seems definitely increased over that of the adults.

S. A. LEVINSON.

THE PHYSIOLOGIC EFFECT OF PHRENIC NEURECTOMY. W. LEMON, Arch. Surg. **14**:345, 1927.

One or both phrenic nerves in dogs were cut, and the effect on respiration was studied by means of an ingenious recording device and the fluoroscope. After five months the dogs were killed and the tissues examined. The operation did not impair the respiratory function although the diaphragm on the side of the cut nerve ceased to function. The diaphragm deprived of its nerve supply atrophied early, and remained practically stationary at a level somewhat higher than normal.

N. ENZER.

THE INFLUENCE OF HYDRAZINE AND ITS DERIVATIVES ON METABOLISM: II. CHANGES IN THE NONPROTEIN NITROGENOUS CONSTITUENTS OF THE BLOOD AND IN THE METABOLISM OF INJECTED GLYCINE IN HYDRAZINE INTOXICATION. H. B. LEWIS and S. IZUME, J. Biol. Chem. **71**:33, 1926.

III. THE MECHANISM OF HYDRAZINE HYPOGLYCEMIA. Ibid. **71**:51, 1926.

The subcutaneous administration of hydrazine sulphate produces a diminution of the normal deamination capacity of the liver, as is indicated by the marked rise in the amino-acid content of the blood following the injections, and in the diminished power of the organism to metabolize injected glycine. The administration of hydrazine may also produce a derangement in the capacity of the liver to synthesize glycogen.

ARTHUR LOCKE.

THE SPECIFIC DYNAMIC ACTION OF GELATIN HYDROLYSATES. D. RAPPORT, J. Biol. Chem. **71**:75, 1926.

When a protein is hydrolyzed by acid or trypsin, in vitro, the properties that cause its peculiar stimulating effect on metabolism remain those of the protein itself, and are to be differentiated from the oxidation-accelerating properties of the constituent amino-acids. The fraction of the protein hydrolysate which contains the active substances was not determined.

CHANGES IN THE INORGANIC CONSTITUENTS OF BLOOD IN SUPRARENALECTOMIZED CATS AND RABBITS. E. J. BAUMANN and S. KURLAND, J. Biol. Chem. **71**:281, 1927.

Suprarenalectomy causes a fall in sodium and chloride content of the plasma. In the cat, a rise of potassium, magnesium, and occasionally of calcium content

in the plasma has been found. The diminution of sodium in the plasma is probably the most important of these observations. In a few other conditions in which injury to the suprarenal occurs, it has been found that a decrease in the sodium and chloride content of the plasma also occurs. The relation of the cations to one another is even more markedly altered in suprarenalectomized animals than the actual amounts of the cations themselves. There is no evidence in these experiments that points to an alkalosis as one of the consequences of suprarenalectomy.

AUTHORS' SUMMARY.

THE NUTRITIVE VALUE OF PLASTEIN. H. H. BEARD, J. Biol. Chem. **71**:477, 1927.

Feeding tests indicate that plastein can replace egg-white as the sole protein in the diet of mice. The plastein was prepared from a peptic digest of egg-white by the method of Wasteneys and Borsook.

(It may be interesting to correlate these two articles with the recent observation of Carrel [*J. Exper. Med.* **44**:503, 1926] that proteoses prepared from peptic digests of fibrin stimulate the growth of cells in pure culture more than embryo juice [whole protein] and much more than peptone and the smaller split products of protein.)

ARTHUR LOCKE.

STUDIES IN BLOOD GLYCOLYSIS. I. KATAYAMA, J. Lab. & Clin. Med. **12**:239, 1926.

The sugar of shed blood gradually decreases on standing without bacterial contamination, and under either aerobic or anaerobic conditions. This decrease is greatest at 38 C. and least in the ice box. Plasma, serum and hemolyzed blood show no loss of sugar on standing. Saturation of whole blood with carbon monoxide does not inhibit glycolysis. When washed blood cells are added to Ringer's solution or to physiologic sodium chloride containing dextrose, fructose or galactose, glycolysis occurs. The decrease in the concentration of sugar is greatest with dextrose and least with galactose. The rate of glycolysis is dependent on the blood cell volume. There appears to be no demonstrable difference between the rates of glycolysis in diabetic and nondiabetic bloods. Furthermore, the blood cells of diabetic bloods cause as rapid a rate of glycolysis in Ringer's solution as the cells of nondiabetic bloods. Insulin therapy or insulin, in vitro, has no effect on the rate of glycolysis. The decrease in the concentration of sugar is accompanied by a production of lactic acid, but the increase in lactic acid does not account for the total amount of sugar lost. Other acids than lactic acid are evidently produced during glycolysis.

S. A. LEVINSON.

THE EFFECT OF DIPHTHERIA TOXIN ON BLOOD SUGAR AND INSULIN ACTION IN RABBITS. R. D. LAWRENCE and O. B. BUCKLEY, Brit. J. Exper. Path. **8**:58, 1927.

It is shown that in rabbits diphtheria toxin in some way leads to reduction or suspension of the action of insulin. This effect is ascribed to the stimulation by the toxin of the thyroid and suprarenal which have an antagonistic influence on insulin.

THE INFLUENCE OF THE CORPUS LUTEUM UPON MENSTRUATION. B. WHITEHOUSE, J. Obst. & Gynec. Brit. Emp. **33**:380, 1926.

Excision of a corpus luteum from the ovary on the eighteenth day of the menstrual cycle was followed by the establishment of menstruation within

two days. The menses had previously been regular. Examinations of the menstrual discharge and of the endometrium curetted two days later showed that typical menstruation had taken place, except that there was no formation of pseudodecidua in the stroma. The next menstrual period did not occur in four weeks, but after a six weeks' interval which fell in with the previously established menstrual date. This showed the menstrual rhythmicity to be independent of the corpus luteum. Whitehouse also aspirated liquor folliculi from the ovaries of three patients at laporatomy, at their midmenstrual interval, and reinjected this into their veins, but was unable to induce menstruation by this procedure. The writer concludes that liquor folliculi does not produce menstrual activity in women, although it has been shown by others to cause estrous in lower animals.

A. J. KOBAK.

INFLUENCE OF VASOMOTOR STATE OF PERIPHERAL BLOOD VESSELS ON LEUKOCYTE CONTENT OF BLOOD. A. F. B. SHAW, J. Path. & Bact. **29**:389, 1926.

The distribution of leukocytes in peripheral blood is influenced by the vasomotor state of the peripheral veins (in rabbits) and "capillaries" (in man). The leukocyte content of peripheral arterial blood is not influenced by vasomotor changes. In addition to the local accumulation of cells in the peripheral vessels under the influence of vasoconstriction, there are "physiologic oscillations" which are independent of vasomotor activity and are systemic in origin. Under prescribed conditions, the local application of cold (man) causes a rise in the leukocytes, and heat locally applied (rabbit) causes a fall in the count.

INFLUENCE OF THYROID ON TEMPERATURE OF RABBITS. V. KORENCHEVSKY, J. Path. & Bact. **29**:461, 1926.

The thyroid plays an important part in the regulation of the body temperature, and therefore its condition has always to be taken into account in considering the resistance to cold or heat, and also in the diseases accompanied by fever.

A COMPARATIVE STUDY OF THE CONTENT OF EPINEPHRINE OF THE SUPRARENAL GLANDS AND ZUCKERKANDL'S ORGANS. DANISCH, Verhandl. d. deutsch. path. Gesellsch. **21**:*222*, 1926.

The sympathetic nervous cells of the chromaffin system in fetus and the new-born are found to a larger extent in the paraganglions (Zuckerkandl's organs) than in the suprarenal glands. The former contain epinephrine from the sixth fetal month, while the latter show this substance only from the seventh month. They contain equal amounts of epinephrine in the tenth month, from which date the production of epinephrine by the suprarenal glands surpasses that of the paraganglions. In extra-uterine life, the epinephrine content of the latter organs decreases gradually in the first year and becomes negative after the second to fifth year. The epinephrine content of the suprarenals rises constantly after a temporary decrease in the first two months of extra-uterine life and reaches its climax in puberty. The small amount of epinephrine found in the suprarenals and paraganglions at stillbirth is the result of an intra-uterine asphyxia causing with increasing duration a gradual exhaustion of epinephrine in these organs.

WILHELM HUEPER.

PHYSIOLOGIC AND PATHOLOGIC CHEMISTRY OF THE TEETH WITH SPECIAL REFERENCE TO THE INFLUENCE OF CALCIUM, VITAMINS AND PARATHYROID GLANDS. GUTTORM TOVERUD, Supp. to Norsk Tandlægeforenings Tidende, 1926, p. 145.

This is the report of an experimental study on rats in the course of which it was found that the chemical composition of the teeth may be influenced by deficiencies of diet and by functional disturbances of the parathyroids.

Pathologic Anatomy

RUPTURE OF THE HEART BY INDIRECT TRAUMA IN A FOUR-YEAR OLD BOY. OTTO SAPLING, Am. J. M. Sc. **173**:353, 1927.

A motor truck passed over the body. The right pubic and iliac bones were broken and there was a rupture, 6.5 cm. long, across the left ventricle, beginning about the midportion of the anterior surface and ending in the lower third of the posterior surface. There was no evidence of injury to the wall of the chest.

PLACENTA CIRCUMVALLATA. J. W. WILLIAMS, Am. J. Obst. & Gynec. **13**:1, 1927.

Williams critically reviews ten theories relating to the cause of this type of placenta, and on the basis of his own extensive studies, he accepts the theory that the chorion frondosum is primarily small and limited. The villi compensate this by proliferating obliquely outward and splitting the adjacent decidua. The condition has no clinical bearing, but represents an interesting anatomic entity.

A. J. KOBAK.

TWO RARE OVARIAN TUMORS. S. WOLFE, Am. J. Gynec. & Obst. **13**:16, 1927.

An adenofibroma of the ovary and a cystic dermoid combined with multiple ovarian fibromas, with their respective clinical histories, are described in detail. Both were associated with fibroid tumors of the uterus.

A. J. KOBAK.

UTERUS DUPLEX BICOLLIS, VAGINA SIMPLEX AND SUPERFECATION. G. L. MOENCH, Am. J. Obst. & Gynec. **13**:60, 1927.

Moench reports a double uterus containing two fetuses, with a difference of apparently from 3 to 4 months in age. Since the corpus luteum inhibits ovulation, superfecation can only occur in the fourth month, when the action of the corpus luteum begins to wane.

A. J. KOBAK.

THE VARIABILITY OF LOCALIZATION OF TUBERCULOSIS IN THE ORGANS OF DIFFERENT ANIMALS: I. QUANTITATIVE RELATIONS IN THE RABBIT, GUINEA-PIG, DOG AND MONKEY. H. J. CORPER and MAX B. LURIE, Am. Rev. Tuberc. **14**:662, 1926.

II. THE IMPORTANCE OF THE DISTRIBUTION OF TUBERCLE BACILLI AS CONCERNS DIFFERENCES OF SUSCEPTIBILITY OF THE ORGANS. H. J. CORPER and MAX B. LURIE, ibid. **14**:680, 1926.

The importance of the deposition of the bacilli in the different organs of the rabbit, guinea-pig, dog and monkey, following the intravenous injection of tubercle bacilli, as this deposition influences the tuberculous involvement of these organs, differs with the species. They may, accordingly be divided into two groups. In one group, the monkey and the guinea-pig, the quantitative

distribution of living tubercle bacilli is of importance for the subsequent tuberculous involvement of the organs. These are the more susceptible animals. In them, the amount of tuberculosis in the different organs is, in a general way, in accord with the amount of tubercle bacilli deposited in a given organ. In the second group of more resistant animals, the rabbit and the dog, individually differing markedly as to the characteristic involvement of the organs, the deposition of large amounts of bacilli in a given organ is not necessarily followed by a correspondingly large amount of tuberculosis. Thus, in the liver of the rabbit, in which there are deposited more bacilli than in the lung and much more than in the kidney, less tuberculosis develops. Again, the spleen of the dog in which there are deposited larger amounts of bacilli than in any other organs of the body, usually escapes macroscopic tuberculosis. But even in the the first group of susceptible animals, the monkey and the guinea-pig, the distribution of tubercle bacilli in the organs is alone insufficient to explain the organic tuberculosis in these animals. It cannot explain the preponderance of the disease in the guinea-pig's lung over its hepatic involvement during the first four weeks after the intravenous inoculation of living tubercle bacilli, in spite of the fact that a far greater quantity of bacilli is deposited in the liver than in the lung. It cannot explain the large size of the tubercles in the lung of the monkey and the occasional absence of tuberculous involvement of the liver, in spite of the fact that the distribution of the bacilli to these two organs is the same as that in the guinea-pig. Therefore, other additional factors must be sought for, in order to account for the susceptibility of the various organs to tuberculosis in the four species of animals under consideration.

H. J. Corper.

Microscopic Changes in Variola. H. E. Michelson and Kano Ikeda, Arch. Dermat. & Syph. **15**:138 (Feb.) 1927.

The literature concerning the etiology of smallpox is briefly discussed. The histology of the lesions of the skin in forty-seven fatal cases. One nonfatal case and two cases in which the patient recovered during the Minneapolis epidemic of 1924-1925, is described in considerable detail. The cases ranged from the third to the twenty-third day of the disease and comprised purpuric, hemorrhagic, confluent and discrete cases. The descriptions show no essential deviations from the classic picture.

R. D. Lillie.

Spontaneous Pneumothorax. A. W. Atwood, Boston M. & S. J. **195**:1237, 1926.

In father and son, both healthy and active men, free from all signs of lung disease, spontaneous pneumothorax occurred, in the father's left lung at the age of 37 and in the right lung at the age of 60 and in the son's right lung at the age of 28.

Combined Acrocephaly and Syndactylism in Mother and Daughter. A. A. Weech, Bull. Johns Hopkins Hosp. **40**:73, 1927.

This is another instance of the familial incidence of acrocephalosyndactylism.

Sickle-Cell Anemia. Hugh W. Josephs, Bull. Johns Hopkins Hosp. **40**:77, 1927.

Experiments are detailed indicating that an unknown substance in the plasma of human blood is absorbed on red cells, which then, in the case of patients with sickle cell anemia, may become altered in shape.

THE RÔLE OF THE CAPILLARIES AND THEIR ENDOTHELIUM IN THE DISTRIBUTION OF COLLOIDAL CARBON BY THE BLOOD STREAM. RICHARD M. BRICKNER, Bull. Johns Hopkins Hosp. **40**:80, 1927.

While small amounts of carbon particles can be demonstrated in some endothelial cells soon after injection, the amount within the Kupffer cells is vastly greater. The endothelium of the marrow, spleen, liver, lymph nodes and possibly the suprarenals differs from other endothelium in being able to take up particles. The probable reasons for this difference are discussed.

THE COURSE OF URANIUM NITRATE INTOXICATIONS IN PREGNANT DOGS. W. DE MACNIDER, SAMUEL T. HELMS and SELINA C. HELMS, Bull. Johns Hopkins Hosp. **40**:145, 1927.

Pregnant dogs are markedly susceptible to uranium nitrate. Some may recover after severe intoxication, while in others the injury to the liver is proportionately more severe than the renal injury; an air hunger type of breathing may develop with coma, convulsions in some and death. The liver and kidney in such animals, as a rule, show no evidences of repair. Severe albuminuria with granular casts occurs, acetone and glucose appear in the urine, the reserve alkali in the blood is reduced and there is retention of blood urea, nonprotein nitrogen and creatine. The clinical course may resemble certain major toxemias of pregnancy.

UNUSUAL ANOMALY OF CORONARY VESSELS IN MALFORMED HEART OF CHILD. R. T. GRANT, Heart **13**:273, 1926.

Blood-filled spaces in the ventricular muscle communicated freely with the cavity of the ventricle and with the coronary vessels. The malformation is interpreted as a persistence and growth of the intertrabecular spaces of the compact myocardium, which normally are reduced to capillaries.

CONGENITAL PERICARDIAL DEFICIENCY. R. T. GRANT, Heart **13**:371, 1926.

A large congenital defect of the pericardium was found in a man, aged 52, dead of carcinoma, who had led an active and hard-working life. The heart itself was small for a man of his height and age, but in keeping with the general emaciation present, and did not show a sign of hypertrophy or dilatation of any chamber. It is concluded that (*a*) absence of the pericardium is not in itself a factor in the causation of cardiac enlargement, and (*b*) under the conditions of normal human life, the pericardium does not play an important part in supporting the heart wall.

THE EFFECT ON THE KIDNEY OF THE LONG CONTINUED ADMINISTRATION OF DIETS CONTAINING AN EXCESS OF CERTAIN FOOD ELEMENTS: I. EXCESS OF PROTEIN AND CYSTINE. T. ADDIS, E. M. MACKAY and L. L. MACKAY, J. Biol. Chem. **71**:139, 1926.

II. EXCESS OF ACID AND OF ALKALI, ibid. **71**:157, 1926.

Rats fed for one third of their lifetime on a diet containing 70 per cent protein grew to the same approximate length as controls, but to a lower body weight, due to the almost complete absence of fat. The weight of the kidneys increased, while that of the liver decreased. No pathologic changes were found in the urine or in the kidneys.

The rate of protein excretion in the urine of rats kept for one third of their lifetime on a diet made acid by the addition of calcium chloride was reduced to about 50 per cent of that of the controls. No pathologic changes

were found in the urine or in the kidneys. Hydronephrosis was found in 30 per cent of the rats fed for a long time on a diet containing 4 per cent of sodium bicarbonate. The urine of these rats was often red with blood and always showed large numbers of erythrocytes on microscopic examination.

ARTHUR LOCKE.

REACTIONS OF BONE MARROW TO INDIA INK. GULLI LINDH MULLER, J. Exper. Med. **45**:399, 1927.

The F. Weber Company drawing ink was used for intravenous injections in rabbits. With large doses, a heavy outpouring of nucleated red cells resulted followed by anemia; with smaller doses, fewer nucleated red cells appeared in the blood and there was increase for a time in hemoglobin and red cells. Pregnancy suppressed these effects. The Berkefeld W. filtrate of the ink had the same effect as the ink itself. In animals injected with ink or filtrates, the marrow showed marked erythrocytic hyperplasia with many open sinuses lined with nucleated red cells. As the dialyzed ink gave practically the same results, and the dialysate proved to be without any effect, the conclusion is drawn that a nondialyzable protective colloid stimulated the hemopoietic organs.

A BACTERIOLOGIC STUDY OF PULMONARY EMBOLISM. EDWARD C. ROSENOW, J. Infect. Dis. **40**:389, 1927.

The conclusion seems warranted that the diplostreptococcus, isolated from postoperative emboli and thrombi, and used experimentally in different animals, is the common cause of postoperative and nonoperative massive thrombosis, leading to fatal pulmonary embolism, and perhaps of closely allied conditions such as infarction of the heart or brain. In the light of these experiments stasis and other factors generally considered as causes of this condition appear to be merely contributory. The immunity found to exist in animals from ten to fourteen days after a series of injections, suggests the possibility of a means of prevention through specific inoculation with a vaccine prepared from this organism.

AUTHOR'S SUMMARY.

TOXIC EFFECTS OF ETHYLENE DIBROMIDE. B. G. H. THOMAS and W. P. YANT, Pub. Health Rep. **42**:370, 1927.

Ethylene dibromide is a component of ethyl gasoline (approximately 2 cc. per gallon of gasoline), as sold at the time of the investigation.

The vapors of ethylene dibromide are highly toxic for guinea-pigs; 0.4 per cent inhaled for one hour produced death within from six to eighteen hours. The minimum lethal dose was not determined. Ethylene dibromide applied on the skin is highly toxic for rats; 0.25 cc. applied on the abdomen produces death within from six to eighteen hours. The minimum lethal dose was not determined. Animals that die as the result of acute ethylene dibromide poisoning have a pathognomonic putrid mushroom odor at autopsy.

Ethylene dibromide is a general poison that produces a granular degeneration of the parenchymal tissue of the kidneys, liver, suprarenals, pancreas, spleen and heart, and produces a swelling and degeneration of the endothelial lining of the vascular system, with a tendency toward a generalized interstitial edema. The most pronounced lesion noted was that of a marked granular degeneration and disintegration of many loops of the convoluted tubules of the kidney.

AUTHORS' SUMMARY.

FOREIGN PARTICLES, THE RETICULO-ENDOTHELIAL SYSTEM AND ANEMIA. A. R. ELVIDGE, J. Path. & Bact. **29**:325, 1926.

Intravenous injections of particles of quartz, of India ink, of carmine or of trypan blue give rise to the appearance of nucleated red cells in the circulation of the rabbit ("blood crises"). The animals recover in the course of two weeks. The nucleated forms begin to appear about five hours after injection. No marked reduction in the red cell count occurs during a "blood crisis." Repeated injections cause a reduction in the red cell count. The degree of the anemia depends partly on the intervals between injections and partly on the doses given. A severe anemia may lead to the death of the animal. Still more immature and embryonic types of nucleated red cells accompany the "blood crises" and anemia. They are most abundant at the peak of the "blood crises." Hemoglobin determinations show the color index to remain around 1 during the course of the anemia. Suspensions of silicic acid gel and ultramicroscopic particles of quartz were injected without result, which speaks against a silicon poisoning effect. The phenomena in question are due to the physical rather than to the chemical nature of the substances injected. They exert their action on cells of the reticulo-endothelial system.

AUTHOR'S SUMMARY.

EXPERIMENTAL URIC ACID NEPHRITIS. J. SHAW DUNN and C. J. POLSON, J. Path. & Bact. **29**:337, 1926.

The intravenous administration of from 0.5 to 1 Gm. of uric acid per kilogram in the form of lithium monourate to rabbits produces necrosis of distal segments of the renal tubules, while the proximal segments are undamaged. The localization of the lesion appears to be determined by precipitation of the uric acid in the distal segments. This renal lesion causes oliguria and diminution of the urea-concentrating function, and there is marked retention of urea in the blood. Continued administration of smaller doses (from 0.025 to 0.1 Gm. per kilogram produces only minor results without cumulative effect.

AUTHORS' SUMMARY.

ATHEROMA OF AORTA. J. B. DUGUID, J. Path. & Bact. **29**:371, 1926.

Atheroma is a condition brought about by impairment of the elasticity and pliability of the tissues of the intima. The lesions, which are marked by the presence of fatty deposit, are the result of the diastolic recoil of the vessel causing the intima to form folds, and the more prominent parts of these folds tend to separate themselves from the mediums. Proliferation of fibrous connective tissue occurs in association with fatty deposits in the tissues of the intima and follows as a reinforcing or reparative measure when this separation takes place. Hyperplasia of fibrous connective tissue tends to diminish the pliability of the intima. If it is irregular in its distribution, it is likely to lead to injury, so that excessive proliferation may bring about further changes of the very nature it is designed to counteract. Atheroma is the mark of a vicious and progressive cycle of such changes. The variations in the appearance seen in atheroma are to be accounted for by the variation in the degrees of elasticity which may be present in different aortas. Factors which tend to increase the range of pulse movements increase the liability to atheromatous changes, and of these factors the most important are hypertension with high blood pressure (hyperpiesis), and the strain caused by the focal fixation of the aorta by its branches. The cicatricial fibrosis of syphilitic

aortitis causes impairment of the intimal pliability and this leads to atheromatous change at the affected parts. Fibrosis cannot be demonstrated in the earliest stages of atheroma, but edema and hyaline change can be seen which may cause considerable loss of pliability in the intima. Early atheromatous changes and acute general toxemic conditions are united in their association with hyaline and edematous change of the aortic intima.

AUTHOR'S SUMMARY.

THE DIURNAL TIDES OF THE LEUKOCYTES OF MAN. A. F. BERNARD SHAW, J. Path. & Bact. **30**:1, 1927.

The author studied and plotted curves of leukocyte counts at intervals of fifteen minutes, the examinations continuing over periods of from two to three hours. During a twenty-four hour day he found that the total leukocytes of a person exhibit two tides each occupying about twelve hours. In general, there is a rise of from 1,000 to 2,000 in the early afternoon followed by a fall and later a greater rise in the middle of the night. Oscillations occur at intervals of fifteen minutes, and the tidal waves are the summation of these rapid oscillations. Food, exercise and sleep do not appear to influence the day and night tides. The curve of the polymorphonuclear neutrophil leukocytes follows closely the course of the tide and is mainly responsible for its character while the lymphocytes and other cells fail to follow the tide. The leukocyte count in a person varies as much in twenty-four hours as do the individual counts of many healthy persons differ one from another. Shaw found a uniform distribution of the leukocytes in the peripheral and central circulations. He also found occasional myelocytes, normoblasts and polychromatophilic red cells in the blood of healthy persons.

E. M. HALL.

THROMBO-ANGEITIS OBLITERANS. J. MARNOCH and EDITH MACRAE, J. Path. & Bact. **30**:39, 1927.

A typical case of Buerger's disease in an Englishman, aged 59, is reported. The patient presented coldness of the extremities, which was more marked on one side, pain on walking, blotchy erythema and cyanosis of one foot and leg. Following the loss of the little and great toes, the leg had to be amputated above the knee. Examination of the vessels in the amputated leg showed obstruction of the popliteal and terminal two thirds of anterior and posterior tibials and practically complete obliteration of the peroneal. Histologic examination revealed marked thickening of the walls of the obstructed arteries and the formation of a thrombus with organization.

E. M. HALL.

SECRETION AND THE GOLGI APPARATUS IN THE CELLS OF THE ISLETS OF LANGERHANS. R. J. LUDFORD and W. CRAMER, Proc. Roy. Soc. Med. **101**:16, 1927.

The appearance in the cells of the islets of Langerhans in normal animals indicate that the cells of the islets in the same animals are at different stages of functional activity, and that this holds good even for the groups of cells in one and the same islet, so that at any one instant the various islet cells are exhibiting various phases of secretory activity. The condiiton in which the most definite and uniform change has been observed is during pregnancy, when the islet cells are particularly active. A similar, but less uniform and less definite change has been found after exposure to heat. A pictographic schema of the secretory cycle in an islet cell is given.

AUTHORS' SUMMARY.

CHANGES IN THE OVARY OF A MOUSE FOLLOWING EXPOSURE TO X-RAYS. F. BROMELL, A. PARKES and U. FIELDING, Proc. Roy. Soc. Med. **101**:29, 1927.

The authors exposed forty-seven female mice, 3 weeks of age, to a full sterility dose of the roentgen ray and found that irradiation caused degeneration of all the oocytes. The membrana granulosa and theca interna, when it was possible to differentiate them, likewise showed degenerative changes. In a few cases, it was noted that the larger follicles became filled with blood and formed cysts; or the cells of the theca interna and membrana granulosa invaded the follicle cavity and formed a corpus luteum atreticum, which persisted indefinitely but appeared to have no effect on the estrous cycle. Simultaneously with these changes the old interfollicular tissue atrophied and the germinal epithelium proliferated, forming epithelial cords. In the adult animals, the ovaries were composed almost entirely of this first proliferation, which tended to become like luteal tissue in some of the cases. The cords of the second proliferation appeared to have no effect on the estric cycle. A. J. KOBAK.

A CASE OF SICKLE CELL ANEMIA IN THE SUDAN. R. G. ARCHIBALD, Tr. Roy. Soc. Trop. Med. & Hyg. **19**:389, 1926.

A case of anemia associated with marked debility, pyrexia, slight jaundice, edema of the feet, enlargement of the liver and lymphatic glands, occurring in a native of the Sudan is described. There was a diminution of the red blood cells, leukocytosis, low hemoglobin percentage, a color index of 0.9, erythroblasts and phagocytosis of red blood cells. The most striking feature in fresh and stained preparations of the blood was the large number of elongated and sickle-shaped erythrocytes, averaging nearly 50 per cent. Hanging-drop preparations of fresh blood suitably sealed and kept at room temperature (31 C.) showed, after twenty-four hours, nearly 100 per cent of sickle cells. The sickling is apparently due to some inherent property of the cell, independent of any substance in the serum. The blood picture is identical with that of sickle cell anemia, a specific entity investigated by American writers. Clinical characters of the case corresponded to those of sickle cell anemia, differing only in the absence of lesion of the skin.

HYPOPHYSEAL GUMMA. A. ESSER, Centralbl. f. allg. Pathol. u. path. Anat. **39**: 97, 1927.

A woman, aged 66, in whom signs of acromegaly had developed recently, entered the hospital because of cardiac decompensation. Syphilitic aortitis (?) and tumor of the hypophysis were diagnosed. Postmortem examination revealed syphilitic mesa-ortitis, two calcified pea-sized nodules of the cortex of the brain, and a gumma of the hypophysis, which weighed 1,500 mg. In the anterior lobe was a small eosinophilic adenoma and near its junction with the posterior lobe was a marked increase in interstitial tissue, mostly hyalinzed and containing remnants of glands, and lymphocytes in groups about typical Langhans giant cells and in rings about many of the small blood vessels. In the midzone and posterior lobe were small colloid cysts. Spirochetes and tubercle bacilli were not demonstrable. The calcified nodules were regarded as healed gummas. Tuberculous lesions were lacking in all organs. Following the tabulation and discussion of twenty reported hypophyseal gummas, in one of which spirochetes were found, Esser concluded he was dealing with a gumma in the course of an acquired syphilis but which had many of the characteristics more often seen in congenital syphilis. GEORGE RUKSTINAT.

THE HISTOLOGY OF OSTEOMYELITIS INFECTIOSA. F. ORSOS, Verhandl. d. deutsch. path. Gesellsch. **21**:110, 1926.

The development of demarcation in osteomyelitis infectiosa was studied. In the first stage the fresh emboli of cocci are surrounded by a small zone of degenerated parenchymatous cells of the marrow and erythrocytes. In the further course the degeneration and necrosis of marrow involves the whole diameter of marrow cavity; the erythrocytes are hemolyzed, an irregular fibrinous network appears and cocci are found in large masses in and around cells still showing nuclear staining. In the next step the marrow at some distance from the primary focus appears hyperemic, the venous sinuses are distended and regenerative myeloic foci are found between them embedded in a serous exudate. An almost unchanged zone separates this area from a territory showing a dense infiltration. It is divided into two layers. In the outer layer proliferating reticulum cells (macrophages) and leukocytes, which become more frequent toward the inner zone, are found. The intermediary zone is formed by degenerated and swollen macrophages. The inner layer is also densely infiltrated, but mainly composed of degenerated cells. In its peripheral part a large number of cocci partly invading the better preserved tissue of the outer layer are present. Then follows again a zone of necrotic fat tissue not showing any reactive processes. Finally, the primary infecting focus is encountered located in necrotic fat tissue containing faintly staining clumps of cocci. The course of the demarcation as illustrated by these alterations is as follows: The cocci in the primary focus die from lack of food and the effect of accumulated toxic products. The raction of the bone marrow against the invaders does not take place in the directly adjacent tissue — it is unable to react on account of exhaustion or saturation with bacterial toxins — but in the undamaged tissue in some distance from the focus. This cellular zone is then invaded by cocci, because they find here a favorable culture medium. On account of this invasion, the cells in the inner layer of the infiltration zone die and a new infiltration zone is formed in some distance, again leaving an unchanged zone between the cocci and the border line. Abscesses are formed when the infecting germs are of a minor virulence and the marrow has time to check the progress of the bacteria by building up a dense capsule of proliferating reticulum cells with a wide infiltration zone behind it. In larger abscesses the typical structure of the demarcation zone disappears and a callous connective tissue takes its place. The number of myeloblasts, plasma cells, macrophages and reticulum cells is increased in the marrow around the abscess, while the specific marrow cells disappear. Moreover, the fat cells degenerate and small cavities filled with a fatty fluid or macrophages containing fat droplets are formed. The fibrillar network becomes denser and the wall of the venous sinuses are thicker thereby. The demarcation of dead tissue in the compact bone takes place in the surrounding living bone starting with resorption of bone on its inner and outer surface, the surfaces of the Haversian canals and newly formed perforating canals. Moreover, an irregular network of narrow fissures appears in the bone. The cocci are present in these fissures and the lacunae of pieces of dead bone. Leukocytic infiltrations in the Haversian canals attempt to stop a further progress of the bacteria. Similar alterations are observed in the cartilaginous tissue in infection. Two types of calcification are seen in the tissue of the proliferated periosteum. The bundles of Sharpey's fibrils are calcified directly, while the reticular tissue forms a spongy matrix and its cells furnish the osteocytes.

W. HUEPER.

A Tumor-Like Osteomyelitis. Von Albertini, Verhandl. d. deutsch. path. Gesellsch. **21**:131, 1926.

In a case of a secondary osteomyelitis of the femur after a prepatellar bursitis, there was a central abscess surrounded by granulation tissue containing many spindle cells and giant cells, resembling in this respect the giant cell tumors of fibrous osteitis.

W. Hueper.

Experimental Inflammation in Anaphylactic Shock. J. Tannenberg, Verhandl. d. deutsch. path. Gesellsch. **21**:144, 1926.

Tannenberg could not substantiate the observations of Auer on animals in protracted anaphylactic shock which showed a much more severe inflammatory reaction of the skin after a rubbing with xylol than the control animals. Tannenberg who used cantharidin found just a reverse reaction, the sensitized animals showing a milder reaction than the normal. He concludes that an increased tonus of the arteries in protracted anaphylactic shock interferes with an intensive inflammatory reaction and that there is not any evidence of a specific localized anaphylaxia.

W. Hueper.

Experiments on the Reaction of Cells. M. Schmidtmann, Verhandl. d. deutsch. path. Gesellsch. **21**:146, 1926.

The p_H of normal cells varies in the different kinds of cells between 6 and 8; but every special cell type has its definite specific p_H. This can be changed only a little (0.2 to 0.5) owing to the buffer action of the cell without interference with its life. Alkalinized cells are small, translucent and distinct in outlines, while acidified cells are large, turbid and indistinct in outlines, and may contain lipoid granules. These structural alterations occur before the cellular reaction changes and are due to changes in the reaction of the environmental fluid.

W. Hueper.

Swelling and Other Changes of the Connective Tissue. A. Dietrich, Verhandl. d. deutsch. path. Gesellsch. **21**:156, 1926.

Dietrich, working with umbilical cord, tendon and mesentery, found that swelling takes place first in the jelly-like substances. In alkaline medium the more liquid, younger jellies swell first and more markedly, while in acid medium the solid jellies show the greatest swelling, beginning with the swelling of the cement substance of the fibrillar bundles. The apparent antagonism is due to the different amount and character of the jelly-like substances in the different kinds of connective tissue. Edema is produced by a liquefaction of the undifferentiated matrix, which is followed by a liquefaction of the less solid jelly-like substances, and finally by a swelling of the fibrils in extreme stages. Other changes of the connective tissue caused by alterations of the colloidal elements are xerosis, myxomatous changes, hyalinization, amyloidosis, transformation of loose connective tissue into strong connective tissue and organization of fibrinous exudates.

W. Hueper.

The Effect of Ultraviolet Rays on the Regeneration Process of the Skin of the Rabbit. A. Abrikossoff, Verhandl. d. deutsch. path. Gesellsch. **21**:163, 1926.

The hyperplastic processes of the epidermis cells after radiation with ultraviolet rays were studied. The radiated area becomes first warmer and redder than the adjacent skin, then the epithelium scales in delicate membranes, and

finally, from three to four weeks after radiation, an increased number of hairs appears in this area. The histologic examination reveals degenerative processes in the epidermis extending into the subcutaneous tissue, while the hair follicles are little affected. After the demarcation of the degenerated part by a wall of leukocytes a regenerative proliferation is observed. The number of the layers of the epithelium of the skin and of the hair follicles is increased. There is a production of giant hairs and of a larger number of lanugo hairs. The epithelization of ulcers is considerably hastened by radiation with ultraviolet rays. The newly formed epithelial lining is thickened and sends solid epithelial processes into the subcutaneous tissue. New hairs are formed in these processes. After the repeated radiation of an artificial ulcer the healing is protracted and epithelial cell proliferations are seen resembling in structure those present in basal cell cancer.

W. HUEPER.

ATROPHIC SCLEROSIS OF SUPRARENAL CORTEX AND OF THYROID WITH ADDISON'S DISEASE. M. B. SCHMIDT, Verhandl. d. deutsch. path. Gesellsch. **21**:212, 1926.

Two cases of simultaneous atrophic sclerosis of the cortex of the suprarenals and of the thyroid gland, with secondary diffuse lymphocytic infiltrations are reported. The symptoms were those of an Addison's disease. Signs of deficient thyroid function were not present. The etiology could not be determined.

W. HUEPER.

SCLEROSIS OF THE PULMONARY ARTERY IN AN INFANT, WITH TRANSPOSITION OF LARGE VESSELS. WATJEN, Verhandl. d. deutsch. path. Gesellsch. **21**:259, 1926.

A case of isolated sclerosis of the pulmonary artery in an infant, aged 6 months, is reported. A transposition of the large vessels, a large defect in the auricular and ventricular septum and an open foramen ovale were seen. The stem of the pulmonary artery and the intrapulmonal and extrapulmonal branches were aneurismally dilated. The wall of the larger and median sized branches was markedly hypertrophic and the intima was thickened. These changes were still more marked in the small and smallest branches, where also deposits of lipoids were present. The functional-mechanical strain to which the wall of the pulmonary artery was exposed continuously, getting its blood from the left ventricle, was regarded as the cause of the pathologic changes. Their rapid development was remarkable. The Wassermann reaction was negative.

W. HUEPER.

WIDESPREAD METASTASES FROM SMALL PRIMARY CARCINOMAS. S. DERISCHANOFF, Virchows Arch. f. path. Anat. **261**:384, 1926.

The author reports three cases of widespread carcinomatosis, in each of which the detection of the pea-sized primary tumor required diligent search. In two cases, malignant proliferation of the mammary duct epithelium and in the third a small, solid, cellular nodule of the thyroid were held responsible for the metastases. In one of the mammary cases and in the thyroid case there was widespread involvement of the osseous system. Metastasis of the bone in the absence of a readily detectable primary tumor should lead to careful examination of the mammary gland and thyroid for the origin of the neoplasm.

O. T. SCHULTZ.

TRANSPLANTATION OF OVARIAN TISSUE INTO TESTIS. H. E. Voss, Virchows Arch. f. path. Anat. **261**:425, 1926.

Voss made a histologic study of thirty-two testes of guinea-pigs into which transplantations of the ovarian tissue of the guinea-pig had been made, the duration of time between transplantation and removal of the organ for examination varying from eighteen to two hundred and fifty-nine days. During the third week after transplantation, lymphocytic infiltration was present about the implant and the center of the latter became fibrotic. The formation of bone, by indirect metaplasia of connective tissue, occurred in seven of the implants. Mitosis was not seen in the covering germinal ovarian epithelium, but the author, accepting the formation of oocytes from germinal epithelium in postembryonic life, thinks it probable that new formation of oocytes occurred. The number of developing follicles seen in the implanted tissue appeared to bear a relation to the nutritional condition of the germinal epithelium. The number of follicles with a compact granulosa layer and an eccentric cumulus, termed secondary follicles by the author, was proportional to the number of young, so-called primary follicles. Large ripe follicles, termed tertiary, were seen in fourteen of twenty-seven experiments. Maturation division of oocytes was seen twice. Corpus luteum formation did not occur. The testicular tissue showed little change, but occasionally desquamation of tubercular epithelium with atrophy of tubules was noted. No changes occurred in the testicular interstitial tissue. The cessation of development of the ovarian follicles beyond the so-called primary or secondary stage is interpreted by the author as evidence of hormonal antagonism exerted by the testis on the implanted ovary. In thirteen of fourteen experiments in which ripe tertiary follicles were formed, a positive ovarian hormonal action was indicated by hypertrophy of the mammary tissue and of the nipples.

O. T. SCHULTZ.

MICROMYELOBLASTIC LEUKEMIA. G. SEEMANN, Virchows Arch. f. path. Anat. **261**:533, 1926.

Seemann reports a case of leukemia in a woman, aged 35, which was unusual because of the formation of tumor-like masses in the mediastinum and left mamma. Ninety-one per cent of the leukocytes of the circulating blood were suggestively lymphocytic in morphology and did not give the oxidase reaction. In the tumor-like masses and in the lymph nodes were many oxidase positive myelocytes and many promyelocytes, with transitions to the oxidase negative lymphocyte-like cells of the blood. The change in the spleen was of the myeloid rather than of the lymphoid type. The cells of the circulating blood were therefore considered to have been micromyeloblasts and the case was believed to be one of myeloblastic leukemia.

O. T. SCHULTZ.

ELASTICITY AND VOLUME OF THE HUMAN AORTA. M. I. HWILIWITZKAJA, Virchows Arch. f. path. Anat. **261**:543, 1926.

The volume of the human aorta removed at post mortem was correlated with the pressure used in filling the aorta and with the amount of fluid displaced after release of the pressure, a smooth curve being obtained when pressure in millimeters of mercury was plotted against the volume. In most of the aortas studied, contractility and irritability of the smooth muscle were found to be preserved, as determined by the reaction of the aortas to epinephrine and electricity. After death the smooth muscle was usually in a state of contraction. In the technical method used by the author, the first

filling of the aorta, which had to overcome the state of contraction of the smooth muscle, gave a lesser coefficient of elasticity than the later distentions, which measured the elasticity due to elastic and collagenous tissue of the wall of the vessel. As a result of his measurements, the author concludes that the distensibility of the aorta decreases progressively with age, the elasticity increasing in proportion to the decrease in distensibility, an observation which indicates a progressive loss of functional capacity with age. Arteriosclerotic changes have a similar effect. The capacity of the aorta increases progressively with age. Those aortas which appeared abnormally narrow on postmortem examination, were found to have a greater degree of distensibility than aortas of apparently normal caliber, indicating that during life the greater distensibility compensates for the narrower lumen and prevents hypertrophy of the left ventricle.

O. T. SCHULTZ.

SCLEROSIS OF THE PORTAL VEIN. T. VITÉ, Virchows Arch. f. path. Anat. **261**: 565, 1926.

Vité describes a case of marked sclerosis of the portal vein with narrowing of the lumen in a man, aged 29, who died as the result of gastric hemorrhage. The vascular change was most marked at the hilum of the liver. The appendix was embedded in fibrous tissue. The author ascribes the portal vein sclerosis and the associated interlobular cirrhosis of the liver to an attack of pylephlebitis which occurred during the acute stage of the appendicitis.

O. T. SCHULTZ.

ECHINOCOCCUS DISEASE OF THE HEART. A. N. DOBROTIN, Virchows Arch. f. path. Anat. **261**:575, 1926.

Three examples of echinococcus disease of the heart are reported. All were in adults. In one case the description is based on a museum preparation and no clinical data are given. In the case seen personally by the author, that of a soldier, aged 45, death occurred suddenly. More than twenty cysts were present in the heart. In the third case, also that of a soldier, cardiac symptoms of progressive severity had been present for twenty-four hours before death. The cyst had ruptured into the pericardium.

O. T. SCHULTZ.

FRAGMENTATION OF THE MYOCARDIUM. H. WILLER, Virchows Arch. f. path. Anat. **261**:586, 1926.

Willer takes issue with Saltykow, who had interpreted the presence of isolated fragmented muscle fibers within the fibrous bands in a case of chronic interstitial myocarditis and of young connective tissue cells and lymphocytes between the fragmented fibers in another case as evidence that the fragmentation had occurred during life. (ARCH. PATH. **1**:270, 1926. Fragmentation occurs chiefly in adults, rarely in children, and never in new-born infants, and it occurs usually in hearts which have been previously damaged. Localized areas of fragmentation about fibrous scars of the myocardium are the result of mechanical action and cannot be interpreted as proof that the fragmentation had occurred during life and had led to fibrosis. The author's observations did not permit him to decide whether fragmentation is an agonal or an intravital process, but he believes that satisfactory evidence of the intravital occurrence of the process has never been adduced.

O. T. SCHULTZ.

Pathologic Chemistry

PENTOSURIA. D. J. LEVY and M. PIERSON, Am. J. Dis. Child. **33**:213, 1927.

A case of pentosuria is described in a child. A reducing substance was found in the urine of a number of the members of the patient's family who had not had any symptoms of diabetes mellitus.

RUTH E. TAYLOR.

THE COMPARISON OF THE CONTENT OF POTASSIUM IN MATERNAL AND PLACENTAL SERUM. H. BAKWIN and H. RIVKIN, Am. J. Obst. & Gynec. **13**:68, 1927.

Bakwin and Rivkin found the average for potassium in placental serum to be 38.5 mg. per hundred cubic centimeters and in maternal serum 20.7 mg. The protein, calcium and chlorides corresponded with the observations of others.

A. J. KOBAK.

LIPID EXCRETION: IV. A STUDY OF THE RELATIONSHIP OF THE BILE TO THE FECAL LIPIDS WITH SPECIAL REFERENCE TO CERTAIN PROBLEMS OF STEROL METABOLISM. W. M. SPERRY, J. Biol. Chem. **71**:351, 1927.

The close similarity in the composition of the excretions of normal dogs and of dogs with bile fistula indicates that the bile is not the source of fecal lipids. A considerably larger amount of lipid is excreted after exclusion of the bile than before.

ARTHUR LOCKE.

A BIOCHEMICAL STUDY OF TOOTH GROWTH. Y. MATSUDA, J. Biol. Chem. **71**:437, 1927.

The lower incisor of the rat is longer, heavier, has a thicker enamel, a greater percentage of ash constituents and a lower percentage of water than the upper incisor. The teeth of the young rat are smaller and contain a lower percentage of ash constituents and a greater percentage of water than the teeth of the older rat. The ash of the lower incisor has a higher percentage of calcium and a lower percentage of magnesium than the upper, but does not differ in the percentage of phosphorus. The ash of the young tooth contains a lower percentage of magnesium than the old tooth but does not differ in the percentage of calcium and phosphorus. Teeth differ from bone in that the calcium and phosphorus of the ash do not change with age.

(No correlation is made of the variation in magnesium content with structure. It may be significant that in the young tooth and in the lower incisor where the magnesium content of the ash is least, the enamel volume tooth volume ratio is greatest.)

ARTHUR LOCKE.

ON THE NATURE OF THE URINE SUGARS. H. S. EAGLE, J. Biol. Chem. **71**:481, 1927.

Glucose is not normally excreted in the urine. What has been considered to be glucose, fermentable by yeast, is in reality a group of substances which gradually decompose only under the conditions of fermentation, and which may be partly removed by a preliminary shaking of the urine with Lloyd's reagent. The kidney interposes an absolute barrier to the elimination of glucose until a certain critical level of blood sugar has been reached which is not normally exceeded, excepting after the administration of upward of 100 Gm. of dextrose.

ARTHUR LOCKE.

THE PURIFICATION OF PEPSIN, ITS PROPERTIES, AND PHYSICAL CHARACTERS. J. C. FORBES, J. Biol. Chem. **71**:559, 1927.

Pepsin may be partially purified by flocculation with safranine followed by a removal of the safranine from the precipitate by extraction with iso-amyl alcohol. The commercial product may be concentrated twenty-two fold in this way with a yield of 65 per cent. The purified preparation is not affected by dry extraction with ether, but is rapidly inactivated when solutions of moderately high hydrogen ion concentration are extracted by alcohol-ether mixtures. It has an iso-electric point of p_H 2.5.

ARTHUR LOCKE.

THE PRESENCE OF AN UNKNOWN SUBSTANCE IN DIABETES INSIPIDUS URINE. A. B. ILLIEVITZ, J. Biol. Chem. **71**:693, 1927.

The urine of a patient who had diabetes insipidus is reported to contain an abnormal substance of unknown composition, purplish and ether-soluble.

ARTHUR LOCKE.

THE PHOTO-ACTIVITY OF THE BRAIN. M. DECRINIS, Klin. Wchnschr. **6**:213, 1927.

The human brain, removed post mortem and exposed to sunlight or quartz light, affects photographic plates. This photo-activity is lost when the tissue is extracted with ether or chloroform, or fixed in formalin.

J. D. WILLEMS.

THE QUANTITATIVE DETERMINATION OF DIASTASE IN THE BLOOD AND URINE IN DISEASES OF THE PANCREAS. R. JANKER, München. med. Wchnschr. **74**:101, 1927.

The pancreatic secretion, diastase, which is present normally in small but constant amounts in the blood and urine, is greatly increased when the pancreatic outflow is obstructed by pathologic processes. A quantitative determination is, therefore, a valuable aid in differential diagnosis.

J. D. WILLEMS.

DISTRIBUTION OF INTRACELLULAR OXIDASES. W. LOELE, Virchows Arch. f. path. Anat. **261**:484, 1926.

By means of the naphthol oxidase reaction, the naphthol and benzidine peroxidase reactions, and the indophenol blue reaction, the distribution of intracellular oxidase was studied in a wide variety of lower animals, mostly species of molluscs. When present, oxidase may occur in surface epithelium, mucus secreting cells, leukocytes and amebocytes. When the naphthol oxidase reaction was positive all the other reactions were also positive, but a negative naphthol reaction might occur when one or more of the other reactions were positive. This is held to indicate that the oxidases have a complex chemical structure, or are mixtures of substances.

O. T. SCHULTZ.

CYSTEINE OF THE CRYSTALLINE LENS. Y. SHOJI, Fukuoka Acta Med. **20**:6 (Jan.) 1927.

The cysteine reaction with the ammonia-nitroprusside (sodium) test of the various tissues of the eye from man, ox, rabbit (albino for choroid etc.) and mouse was strongly positive for the retina and lens, weaker for the choroid, iris, cornea and optic nerve, and negative for the vitreous and aqueous humors

and the sclerotic coat. Even the lead acetate test for sulphur was negative with the aqueous humor. The reaction for cysteine in the lens grew proportionately weaker with the development of cataracts, to a negative result with complete opacity. Dextrose did not affect the reaction, strong oxidizing agents caused a loss of reaction but without signs of opacity, and ultraviolet rays changed dilutions of the crystalline lens so the reaction was partly or completely lost. B crystalline gave a strong cysteine reaction, A crystalline a weaker reaction, and the albuminoid was negative.

Microbiology and Parasitology

PURPURA FULMINANS DUE TO THE MENINGOCOCCUS. S. BATTLEY, Am. J. Dis. Child. **33**:244, 1927.

A fatal case of meningococcus septicemia with purpura, with hemorrhages into the skin, pleura, epicardium, thymus and suprarenals, is described. The organisms were present in the skin and uvula. A review of the literature and his own observations impress the author with the possibility of meningococcus sepsis as the most frequent cause of fulminating purpura.

RUTH E. TAYLOR.

BACTERIA IN NASAL CULTURES FROM RABBITS AND THEIR RELATION TO SNUFFLES AND PNEUMONIA. CARROLL G. BULL and C. M. MCKEE, Am. J. Hyg. **7**:110, 1927.

Snuffles in rabbits may be due to infection of the upper respiratory passages by *Bacterium lepisepticum* or *Bacillus bronchisepticus* or to a mixed infection by both. *Bacterium lepisepticum* causes the more severe form.

AN UNIDENTIFIED BACILLUS FROM NASAL DISCHARGES AND PURULENT INFECTIONS IN RABBITS. CARROLL G. BULL and C. M. MCKEE, Am. J. Hyg. **7**:114, 1927.

This is a small gram-negative bacillus.

THE EFFECT OF ANTISEPTICS ON THE NASAL FLORA OF RABBITS. CARROLL G. BULL and G. HOWARD BAILEY, Am. J. Hyg. **7**:185, 1927.

Solutions of mercurochrome-220 soluble, phenol, dichloramine T, acriflavine and other antiseptics were not able to free the upper respiratory passages of rabbits from pathogenic germs.

THE BACTERIAL CONTENT OF THE UTERUS AT CESARIAN SECTION. J. W. HARRIS and J. H. BROWN, Am. J. Obst. & Gynec. **13**:133, 1927.

Cultures of the contents of fifty uteri were made through the operative incision at cesarian section. The cultures were sterile in twenty-eight patients who had been in labor less than six hours. One patient not yet in labor, but with membranes ruptured for ten hours, yielded a positive culture. In the remaining cases, in which the patients were in labor over six hours, infection was found. In six of the positive cases, the membranes had not yet ruptured. Ascending infection and vaginal manipulations were considered factors in producing the uterine infection. Although premature rupture of the membranes adds materially to the danger of infecting the amniotic fluid, intact membranes did not offer an effective barrier in six cases. The relative merits of classic conservative cesarian section over low cervical section are discussed.

A. J. KOBAK.

THE VARIABILITY OF LOCALIZATION OF TUBERCULOSIS IN THE ORGANS OF DIFFERENT ANIMALS: III. THE IMPORTANCE OF THE GROWTH OF TUBERCLE BACILLI AS DETERMINED BY GASEOUS TENSION. H. J. CORPER, MAX B. LURIE and NAO UYEI, Am. Rev. Tuberc. **15**:65, 1927.

These studies emphasize the importance of slight changes in oxygen tension on the growth of tubercle bacilli. These changes appear significant in the flaring up of old tuberculous lesions in man with resultant progressive and fatal disease, since conditions which can change the oxygen tension in tuberculous foci from 0.7 mm. to 3.8 mm. may be sufficient to change a completely quiescent focus into progressive disease. The studies also make it apparent that changes in bodily conditions which can alter the curve of oxygen dissociation of the blood may exert a marked influence on the progress of the disease. If the flow of oxygen to the tissues is increased, dormant tubercle bacilli in these tissues may multiply. One of the most important single factors influencing the curve of oxygen dissociation of human blood is its state of hydrogen ion dissociation. Conditions such as fatigue and starvation, which tend to increase the hydrogen ion concentration of the blood, and which therefore increase the rate of dissociation of oxyhemoglobin, also influence in a deleterious way for man the progress of the disease, while factors, such as rest and adequate nourishment, which tend to decrease the hydrogen ion concentration of the blood and thus depress the dissociation of oxyhemoglobin, have a beneficial effect on tuberculosis; for thus the oxygen available for the tissues is reduced, and the growth of the bacilli in the tuberculous lesions is retarded.

H. J. CORPER.

THE VARIABILITY OF LOCALIZATION OF TUBERCULOSIS IN THE ORGANS OF DIFFERENT ANIMALS: IV. THE CELLULAR FACTOR IN THE SUSCEPTIBILITY OF THE VARIOUS ORGANS. H. J. CORPER and MAX B. LURIE, Am. Rev. Tuberc. **15**:237, 1927.

One factor in the susceptibility of an organ to tuberculosis is the inherent ability of the cells in which the tubercle originates in a given organ to destroy tubercle bacilli. In the four species of animals studied (guinea-pig, rabbit, dog and monkey) the largest tubercles were formed after the intravenous injection of dead tubercle bacilli into the organ which was the seat of the greatest tuberculous involvement when living bacilli were injected intravenously. In the guinea-pig the spleen becomes most involved after the intravenous injection of living bacilli, and the tubercles resulting from the intravenous injection of dead bacilli are larger than in any other organ of the same animal. The rabbit lung is most involved after the intravenous injection of living bacilli and contains the largest tubercles after the intravenous injection of dead bacilli.

The liver is the most susceptible organ in the dog after intravenous injection with living bacilli, and the tubercles resulting from the intravenous injection of dead bacilli are larger in this organ than in any other organ in the same animal. Species susceptibility to the human and bovine strains of tubercle bacilli is correlated with the chemical constitution of the strain of tubercle bacillus in question, as related to the destructive power which the cells in which the tubercle originates in the various organs of the different animals have for these bacilli. The bacillus of the human type contains less lipoid than the bovine strain, and dead bacilli of the human type are more readily destroyed by the phagocytic cells of the various organs of the rabbit than are dead bacilli of the bovine type. This fact may explain in part the strikingly greater susceptibility of this animal to the bacillus of the bovine type. The

guinea-pig, being susceptible to both strains of tubercle bacilli, but slightly more so to the bovine, supplies partial explanation in that the amount of tuberculosis developed in response to dead bacilli in the various organs is greater with the bovine than with the human strain, but this difference is not so marked as in the rabbit. Finally, the dog is equally susceptible to the bovine and the human strains of living bacilli, and no marked difference in the size of tubercles in the same organs was noted with the dead bacilli. The cell of origin and the nature of the tuberculosis occurring in the different organs of the different animals studied do not differ in kind in the animals given intravenous injections of living, virulent tubercle bacilli of the human or the bovine type from those occurring in animals given intravenous injections of dead tubercle bacilli of either type. The only difference is one of degree.

H. J. Corper.

The Types of Tubercle Bacilli in Human Tuberculosis. Lydia Rabinowitsch-Kempner, Am. Rev. Tuberc. **15**:225, 1927.

Tuberculosis in human adults, especially phthisis pulmonum, in most cases is caused by the typus humanus of the tubercle bacillus. In a great number of cases, however, especially in children, the typus bovinus must also be considered as the cause of even severe infections. Occasionally tuberculosis is caused in man by strains of tubercle bacilli possessing the biologic properties of the avian type. A strict division of the several types is not always possible, as there are atypical intermediate strains. Atypical strains occur frequently in cases of tuberculosis of the skin (lupus). In a case of dermatomyositis recurrens cultures of attenuated bovine tubercle bacilli have been isolated from the associated pulmonary and intestinal foci, and a strain with the biologic properties of avian bacilli from the skin. The possibility is not to be excluded that under certain conditions a transformation of the primary type may take place in the human body. For the definitive decision of the question of type, a further study of the atypical strains of tubercle bacilli is recommended.

H. J. Corper.

Monilia Infection of the Tongue. Erwin R. Zeissler, Arch. Dermat. & Syph. **15**:171 (Feb.) 1927.

Two cases of mycosis of the mouth are described, one due to *Monilia pinoyi* and associated with a nonpathogenic yeast and an unidentified mold. It is pointed out that thrushlike mycosis of the mouth may be caused by a variety of fungi.

Human Infections with the Organism of Contagious Abortion of Cattle and Hogs. Alice C. Evans, J. A. M. A. **88**:630 (Feb. 26) 1927.

A brief summary is given of twenty cases of undulant fever in the United States, all of which have occurred in recent years. By means of the agglutinin absorption test, the infective agent in all these cases was definitely determined to be the *abortus* variety of *Brucella melitensis*.

The twenty cases were distributed in eight states scattered from the Atlantic to the Pacific oceans. They were recognized by ten observers, one of whom discovered seven of the cases. Three of the cases occurred in one family on a ranch in Utah. In two cases it appeared that the infection was contracted by handling carcasses of infected hogs in slaughter houses. Most of the

remaining cases appeared to have been contracted by drinking infected cow's milk, or by taking care of cows that had aborted. A brief review is given of undulant fever contracted from cattle in Italy, South Africa and other countries.

AUTHOR'S SUMMARY.

MICROBIC DISSOCIATION IN VIVO AS ILLUSTRATED BY A CASE OF SUBACUTE SEPTICOPYEMIA. RALPH R. MELLON, J. Bact. **13**:99, 1927.

In a case of suppurative thyroiditis and septicopyemia, aseptically obtained thyroid pus showed a bacterial flora of great pleomorphism. The dominant form was a rather coarse thread or long bacillus which gave rise to coccoid exospores, and these in turn showed varying degrees of segmentation closely approaching a tetrad. Miliary abscesses in the liver showed an identical flora, but in the kidney the abscesses showed only thread forms with no exospores and tetrads. Correspondingly, only the anaerobic threads were obtained by culture from the kidney, while an aerobic micrococcus-tetrad culture was also obtained from the liver and thyroid. From previous demonstrations in vitro that tetrads and micrococci are genetically derived from the exospores of bacilli and threads, the fundamentally composite nature of the flora of this case is explained. The evidence is presumptive, therefore, that the kidney did not contain the coccus because it lacked the environmental essential that the liver and thyroid possessed, whereby the bacillus is forced through a complete life cycle that ends with development of the coccus.

AUTHOR'S SUMMARY.

SOME OBSERVATIONS ON THE LYMPH AND BLOOD VESSELS OF THE RABBIT TRACHEA: WITH PARTICULAR REFERENCE TO THEIR RÔLE IN THE PATHOGENESIS OF EXPERIMENTAL LOBAR PNEUMONIA. BEATRICE CARRIER SEEGAL and DAVID SEEGAL, J. Exper. Med. **45**:203, 1927.

Winternitz, Smith and Robinson have offered as experimental evidence in support of the lymphatic path of infection in lobar pneumonia, a series of injection experiments of the rabbit's tracheal submucosa.

What these investigators supposed to be lymphatics are shown to be an unusual type of venous supply consisting of short, wide and richly anastomosing vascular channels.

There is little homology between the structure and distribution of the lymph and blood vessels in the human and rabbit trachea.

Injections of pneumococci by the tracheal route to produce lobar pneumonia in the rabbit include an intravenous administration of these organisms when the mucosa is pierced or injured.

AUTHORS' SUMMARY.

EXPERIMENTAL EPIDEMIOLOGY OF TUBERCULOSIS. DAVID PERLA, J. Exper. Med. **45**:209, 1927.

Normal guinea-pigs exposed to tuberculous cage mates infected intraperitoneally and without cutaneous ulceration readily contract spontaneous tuberculosis. The incidence of spontaneous tuberculosis increases with the intensity and with the duration of exposure. Spontaneous tuberculosis acquired from infected cage mates has with few exceptions the characters of an infection that has entered by way of the digestive tract, disease of mesenteric and cervical lymph nodes being conspicuous. Guinea-pigs exposed to tuberculous animals in the same room but not in the same cage may acquire tuberculosis that has the characters of a bronchiogenic infection associated with lesions of the lungs and tracheobronchial lymph nodes.

AUTHOR'S SUMMARY.

A Filtrable Yeastlike Microorganism (Shizosaccharomyces Filtrans, n. sp.). Paul A. Lewis, J. Exper. Med. **45**:277, 1927.

A micro-organism cultivated in the course of experiments with the virus of hog cholera shows the following points of interest. It is pleomorphic in extreme degree. Among the forms are coccoid and bacillary bodies at the lower limits of visibility. The culture can be completely regenerated after filtration through some Berkefeld N filters although these retain *Bacillus abortus* Bang. The culture is carried forward by multiplication of forms of easy visibility (1.5 microns). Associated with these at times are still larger forms bearing a striking general resemblance to those yeasts which develop abortive mycelia. Motile bacillary forms appear to arise as buds or branches from round or ovoid cocci. While cognizance is taken of possible alternatives the micro-organism is provisionally classified with the yeasts and in recognition of its filtrability is named *Schizosaccharomyces filtrans.* The culture is not pathogenic for swine.

Author's Summary.

The Histological Expression of the Natural Resistance of Rabbits to Infection with Human and Bovine Type Tubercle Bacilli. Paul A. Lewis and Everett S. Sanderson, J. Exper. Med. **45**:291, 1927.

The natural resistance of the rabbit to infection with the tubercle bacillus of human type is apparently referable to a failure of this type of bacillus to multiply in the body of this species to any considerable extent. Exceptionally there are localized lesions (kidneys and lung nodules) which are associated with abundant growth and show that this failure to multiply may be due to some positive growth restraining factor rather than to a failure of suitable nutritive materials. There is no qualitative difference in the cellular response to the bacillus of human type contrasted with that to the bovine type, that seems adequate to account for the difference in susceptibility. The eventually more abundant tubercle formation and the degenerative changes occurring in the tubercles formed with the bovine type bacillus seem to be a consequence of the abundant growth of this type; the initial response to equal quantities of the two when injected intravenously being approximately the same, both quantitatively and qualitatively.

Authors' Summary.

The Relation Between Invasion of the Digestive Tract by Paratyphoid Bacilli and Disease. Theobald Smith and Helena A. M. Tibbetts, J. Exper. Med. **45**:337, 1927.

Hog cholera bacilli fed to mice disappear from the stomach within twenty-four hours, but remain and perhaps multiply in the ileum for at least several weeks. They promptly penetrate the mucosa and may be found in the spleen. Bacilli introduced subcutaneously quickly pass into the intestinal tract where they may be found for some weeks. Infected mice may harbor bacilli in the spleen for several months. Mice possess a relatively high degree of natural resistance toward hog cholera bacilli which gives way to large doses. Disease is probably the result of the invasion of the viscera from the digestive tract following feeding, but the relation between the dose fed and the numbers penetrating the mucosa is variable and the conditions favoring such invasion are not determined. Contact with mice discharging bacilli failed to cause recognizable invasion of the digestive tract or the viscera.

Authors' Summary.

STUDIES ON A PARATYPHOID INFECTION IN GUINEA-PIGS. JOHN B. NELSON and THEOBALD SMITH, J. Exper. Med. **45**:365 and 377, 1927.

After describing the course of a natural paratyphoid outbreak in a guinea-pig stock, the factors bearing on the maintenance of the endemic state are discussed. The hypothesis is advanced that the epidemic passes into the endemic phase from the weeding out of individuals of low natural resistance with a gradual adjustment of the invader to the population on a lowered level of resistance.

BIOLOGICAL CHARACTERS OF A MUCOID VARIANT OF B. PARATYPHI FROM GUINEA-PIGS. JOHN B. NELSON, J. Exper. Med. **45**:379, 1927.

Two mucoid variants recovered from guinea-pigs spontaneously infected were studied. The mucoid character of growth was constant. It is suggested that it concerned a variant of permanent characters transmitted to the guinea-pig stock with the normal type of *B. paratyphi.*

EPIDEMIC ENCEPHALITIS AND SIMPLE HERPES. SIMON FLEXNER, J. General Physiol. **8**:713, 1927.

The purpose of this paper is to explain the state of the knowledge of the etiology of epidemic encephalitis, and especially to draw a line of demarcation between the established virus of simple herpes and the hypothetical virus of epidemic encephalitis. It has already been shown that the experimental observations on rabbits do not suffice to prove the identity of the virus herpes with that of encephalitis. The discussion in this paper shows that identity cannot be postulated on the basis of the experiments performed on guinea-pigs. Attention has been drawn to the significant fact that there is lack of harmony in the positive results of those investigators who believe that the incitants of epidemic encephalitis have been discovered. An attempt has been made to attribute some of the discrepancies reported by these investigators either to accidental or contaminating microbic agents, or to the uncovering of virulent agents existing in a latent state in the animals employed for inoculation, the existence of which was not previously known or suspected. Since past experience leads us to believe in a single incitant for widespread epidermic diseases, it is probable that, when certainly discovered, the microbe of epidermic encephalitis will prove to be simple and not multiple. The direct corollary to this point is that up to the present the etiology of epidemic encephalitis has not been determined.

AUTHOR'S SUMMARY.

A PATHOGENIC BACILLUS SUBTILIS. W. J. BAIS, J. Infect. Dis. **40**:313, 1927.

A case of gangrene of the lungs is reported with the isolation of *B. subtilis* in cultures from the blood.

AUTHOR'S SUMMARY.

OCCURRENCE OF BACTERIUM COLI OF INTESTINAL ORIGIN ON HANDS OF FOOD HANDLERS. W. A. BUICE, H. C. SEHESTED and R. B. DIENST, J. Infect. Dis. **40**:348, 1927.

Bacterium coli of intestinal origin was present on the hands of food handlers while at work, in 8.38 per cent of the 337 tests made.

AUTHORS' SUMMARY.

FURTHER STUDIES ON THE ISOLATION AND CULTIVATION OF BACTERIUM ABORTUS (BANG). FOREST HUDDLESON, D. E. HASLEY and J. P. TORREY, J. Infect. Dis. **40**:352, 1927.

The growth activity of *Bact. abortus* is governed by an increased carbon dioxide tension, by the liberation of hydrogen sulphide and by the formation of ammonium magnesium phosphate; the production of the latter two substances being controlled by an abundance of carbon dioxide and the reaction of the culture medium.

It is pointed out that there is a biochemical reaction which serves to distinguish *Bact. abortus* from *Br. melitensis*, that is, in the formation of ammonium magnesium phosphate and liberation of hydrogen sulphide.

Bact. abortus may be measured quantitatively in infective milk with a high degree of accuracy by employing, as a culture medium, gentian violet, beef liver infusion agar, and incubating in an atmosphere containing from 5 to 10 per cent carbon dioxide.

In the separation of cream by gravity the rising globules of fat and body cells carry with them the majority of the abortion bacteria in milk.

The culturing of the gravity cream layer of milk is just as efficient for determining the presence of *Bact. abortus* in milk as are guinea-pig inoculation methods.

Bact. abortus does not appear to multiply in milk held at ice-box temperatures and decreases in numbers rapidly when held longer than eight days.

The number of colonies of *Bact. abortus* which develop on plated culture medium decreases as the length of time they are held aerobically at 37 C. increases.

AUTHORS' SUMMARY.

THE EFFECT OF ZINC IN EXPERIMENTAL SYPHILIS. JOHN E. WALKER, J. Infect. Dis. **40**:377, 1927.

Zinc chloride and zinc acetate have therapeutic indexes in experimental syphilis of approximately 1, a result somewhat better than previously obtained by other authors with mercury, and corresponding closely with the effect of simple compounds of vanadium, gold and silver.

AUTHOR'S SUMMARY.

THE RESISTANCE OF DIFFERENT CONCENTRATIONS OF A BACTERIOPHAGE TO ULTRAVIOLET RAYS. RUDOLPH FISHER and EARL B. MCKINLEY, J. Infect. Dis. **40**:399, 1927.

Within the limits of probable error in these experiments, the resistance to ultraviolet rays of this lytic principle is directly proportional to its concentration and appears to be a logarithmic function thereof. Destruction begins promptly and proceeds by a diminution in the number of active particles. The effect is not a photosensitization to heat. If irradiated tubes are observed without shaking, eighteen-hour incubation with *B. coli* D reveals regular differences in turbidity.

AUTHORS' SUMMARY.

BACTERIOLOGIC STUDIES ON BIOLUMINESCENCE. I. CAUSE OF LUMINESCENCE IN THE FRESH WATER SHRIMP, XIPHOCARIDINA COMPRESSA (DE HAAN). YOSHIWO YASAKI, J. Infect. Dis. **40**:404, 1907.

The luminosity of the shrimps in Lake Suwa is due to an infection by luminous bacteria. It may be said that the shrimps are suffering from a "light disease" caused by such infection.

No description of this form of luminous bacterium is found in the literature, and this is probably the first form found in fresh water. This luminous bacterium is named *Microspira phosphoreum* (Yasaki).

AUTHOR'S SUMMARY.

EFFECT OF GENTIAN VIOLET AND MERCUROCHROME ON ENDOMYCES ALBICANS. ESTHER BRIDGMAN CLARK, J. Infect. Dis. **40**:423, 1927.

Gentian violet completely inhibits the growth of the mycelial and yeast forms of endomyces albicans in dilutions up to 1:100,000, and inhibits to some extent in dilutions to 1:400,000. A dilution of 1:10,000 of mercurochrome-220 soluble causes no inhibition in the growth of the mycelia of yeast forms of endomyces albicans.

AUTHOR'S SUMMARY.

FOOD ACCESSORY SUBSTANCES (VITAMINS) AND THE TUBERCLE BACILLUS. NAO UYEI, J. Infect. Dis. **40**:425 and 433, 1927.

Tubercle bacilli fail to grow when seeded with suspensions containing 10 mg. or less of young active bacilli per cubic centimeter, while a suspension containing 50 mg. or more per cubic centimeter grows in Long's synthetic medium containing 2½ per cent agar. On egg mediums growth occurs at lower concentration of suspensions of the bacilli. All attempts to demonstrate the presence of a growth stimulating substance or substances in the cell of tubercle bacilli or in their metabolic products in the form of old tuberculin failed. These results seem to indicate that the "bios" theory of Wildiers for yeast is not applicable for tubercle bacilli as an explanation of their failure to grow when thinly seeded on artificial mediums.

Cod liver oil concentrate ("Oscodal" of Funk and Dubin), known to be rich in fat soluble A vitamin and the antirachitic vitamin, exerts an indifferent action on the growth of tubercle bacilli. Conforming with the results of other investigators with other bacteria, substances rich in vitamin B, namely, Harris' yeast vitamin, orange juice, tomato juice and cabbage juice, exert a pronounced stimulating effect on the growth of tubercle bacilli. With the technic used in these studies, the acceleration of growth is easily discernible in concentration of 1:1,000 for Harris' yeast vitamin, 1:100 for tomato juice and orange juice and 1:20 for cabbage juice.

AUTHOR'S SUMMARY.

THE NECESSITY OF CARBON DIOXIDE FOR THE GROWTH OF BACTERIA, YEASTS AND MOLDS. GEORGE E. ROCKWELL and JOHN H. HIGHBERGER, J. Infect. Dis. **40**:438, 1927.

Inhibition of the growth of several strains of bacteria, mold and yeast, incubated over an alkaline solution is shown. Factors which govern the demonstration of inhibition of growth are: the presence of carbohydrates in the medium, the protein content of the medium, the amount of the inoculum, the reaction of the medium and the concentration of salt in the medium. The opinion is expressed that all bacteria, yeasts and molds require carbon dioxide for their growth, and that the gas is used as a source of carbon.

AUTHORS' SUMMARY.

METAPHEN (4-NITRO-3, 5-BISACETOXYMERCURI-2-CRESOL) AND ITS BACTERICIDAL PROPERTIES. GEORGE W. RAIZISS and M. SEVERAC, J. Infect. Dis. **40**:447, 1927.

Metaphen possesses unusually high bactericidal properties, especially when left in contact with the micro-organisms for more than one hour, in which

respect it is more effective than any of the other germicides we have employed. It is almost four times less toxic when injected intramuscularly than when injected intravenously. The toxicity when given by mouth is also comparatively low. It is nonirritating in therapeutic dilutions. Experimental work on animals, as well as clinical observations, confirm its high efficiency as a bactericide.

AUTHORS' SUMMARY.

EFFECT OF BACILLUS ACIDOPHILUS ON INTESTINAL PUTREFACTION AND INDICAN OUTPUT. M. KAHN, J. Lab. & Clin. Med. **12**:226, 1926.

After ingestion of *Bacillus acidophilus* for from three to five days, this organism can be found in the feces in large numbers if the culture is given to the patient together with lactose; and in smaller numbers if given by itself. With the appearance of the micro-organism in the feces, the output of urinary indican decreased in most cases, indicating a decrease in the extent of intestinal putrefaction. There is evidence which seems to show that in the formation of the ethereal sulphates, two opposite effects occur in connection with the ingestion of *Bacillus acidophilus;* a decrease of the "intestinal" ethereal sulphates, caused by the decrease of intestinal putrefactive processes, and an increase of the "metabolic" ethereal sulphates, caused by lessened formation of indican.

S. A. LEVINSON.

SEPARATION OF VIRULENT CULTURES OF B. DIPHTHERIAE INTO VIRULENT AND AVIRULENT TYPES. MARY L. COWAN, Brit. J. Exper. Path. **8**:6, 1927.

The author succeeded in separating an avirulent, nontoxicogenic type ("R") from a virulent culture of *B. diphtheriae.*

STREPTOCOCCAL HEMOLYSIN AND ANTAGONISTIC ACTION OF BILE AND BILE-SALTS JOHN GORDON, Brit. J. Exper. Path. **8**:38, 1927.

The action of streptolysin is inhibited by bile and bile-salts. This neutralization is difficult to reconcile with the theory that pernicious anemia is caused by streptococci in the duodenum.

A COMPARISON OF THE PATHOGENICITY FOR MICE OF THE NORMAL AND ROUGH FORMS OF SALMONELLA ENTERITIDIS (GÄRTNER). A. N. GOYLE, J. Path. & Bact. **29**:365, 1926.

The N (=H) form of *B. enteritidis* of Gärtner is much more virulent for mice than the R form of the same strain. The filtrate of the autolyzed culture of the N is also more toxic than that of the R form. As shown by Cathcart, heating to 100 C. reduces, but does not destroy the toxicity of the filtrates of the N form.

AUTHOR'S SUMMARY.

LARGE MONONUCLEAR LEUKOCYTOSIS IN RABBITS IN INFECTION WITH BACT. MONOCYTOGENES. E. G. D. MURRAY, R. A. WEBB and M. B. R SWANN, J. Path. & Bact. **29**:407, 1926.

In investigating a spontaneous epidemic disease of rabbits, a micro-organism was isolated in pure cultures which reproduced the characteristic lesions of the natural disease. The bacteriologic characters of this bacillus are described and the impossibility of identifying it with previously recorded organisms justifies its being considered a new species. The name *Bacterium monocytogenes*

is proposed for this organism because it is apparently a specific stimulant for the large mononuclear cells of the blood. At any rate, the main cellular reaction induced by the organisms is almost exclusively mononuclear.

RESEARCH ON RABIES. C. LEVADITI, S. NICOLAU and R. SCHOEN, Ann. de l'inst. Pasteur **40**:973, 1926.

The virus of rabies is classed with the protozoa in the family of *Glugeides* and the name *Glugea lyssae* is used. This virus has a complex life cycle, of which only certain phases, the intracellular pansporoblastic phase and the ameboid and spore phase which is filtrable, are known. The "fixed virus" (of a fixed virulence for a given animal species after passage) is a variation created by mutation, and neurotropic strictly for the animal which has served in the mutation. Multiple forms of *Glugea lyssae* probably exist in nature.

G. B. RHODES.

THE CECAL SPIROCHETES. G. SANARELLI, Ann. de l'inst. Pasteur **41**:1, 1927.

Two strains of spirochetes were isolated from guinea-pigs that died following obscure infections of intestinal origin. One strain was obtained from the heart blood and one from splenic tissue. They are facultative anaerobes, delicate, though capable of cultivation on plain agar, and of a low degree of virulence.

G. B. RHODES.

RESEARCH ON FILTRABLE FORMS OF BACTERIA. E. BURNET, Arch. de l'inst. Pasteur de Tunis **15**:292, 1926.

The macerated organs of guinea-pigs and mice that died from inoculations of *Bacillus pestis* were filtered through Chamberland filters L_1, L_2, L_3, with the result that in four cases either cultures were obtained or animals were infected from the filtrate. The author concludes that filtrable form of *B. pestis* exist, originating in lytic disintegration of organisms in tissues.

G. B. RHODES.

CONCERNING THE NONACID-FAST STAGE OF THE TUBERCLE BACILLUS. J. REENSTIERNA, Arch. de l'inst. Pasteur de Tunis **15**:309, 1926.

A study of yeastlike organisms from the sputum and blood of tuberculous subjects and of similar organisms originating in pure cultures (single cell isolation) of the tubercle bacillus leads to the supposition that these forms are nonacid-fast forms of the tubercle bacillus, and probably saphrophytes of the oidium group which exceptionally can become pathogenic. Complement fixation was obtained with these forms as the antigen and the serum of tuberculous patients. The yeastlike cultures were not pathogenic for guinea-pigs.

G. B. RHODES.

SIGNIFICANCE OF THE SEDIMENTATION REACTION FOR THE STATISTICS IN SANATORIUMS. W. SCHNIPPENKÖTTER, Beitr. z. klin. d. Tuberk. **65**:85, 1926.

It is recommended that the results of systematic sedimentation reactions be used as an additional factor in the statistics to show the results of sanatorium treatment. In combination with the classification, type of lesion, temperature and the bacillary observations, the sedimentation reaction may give added information.

MAX PINNER.

How Can Reactions Following "Sanocrysin" Medication be Explained? A. Beekmann, Beitr. z. Klin. d. Tuberk. **65**:130, 1926.

In addition to the therapeutic effects of "Sanocrysin," the following results may be observed after experimental or therapeutic administration:

1. General nervous symptoms, some of which may be observed immediately after the injection, such as nausea, vomiting, congestion, palpitation and dyspnea.

2. Increase in temperature, which may last for a long time and which does not seem to depend on the size of the dosage.

3. Albuminuria, which again does not depend on the size of the dosage.

4. Inflammatory reactions in the skin, mucous membranes and serous membranes. The latter may occur without the tuberculous focus in or near the site of involvement.

5. Disturbances in the gastro-intestinal tract.

6. Alterations in the blood picture, particularly an extreme eosinophilia which reached 42 per cent in one of the patients who had absolute leukopenia. In an attempt to explain these reactions, it is emphasized that neither Møllgaard's theory of tuberculin shock, nor Feldt's theory that the gold acts specifically on the tuberculous tissue, nor Bang's theory that all toxic symptoms are a manifestation of metallic poisoning, can fully explain the phenomena, particularly those which are acute after the injections. The author believes that the late symptoms are due to metallic poisoning, whereas the immediate reactions are phenomena of anaphylaxis, since they practically never occur except after the first injection. Observation of a patient who reacted violently after the first injection and who had worked years before in contact with gold solutions in an industrial plant and the observations on eosinophilia support the author's theory.

Max Pinner.

Reinfection with Tuberculosis in Man. A. Ghon, Verh. d. Deutsch. path. Gesellsch. **21**:328, 1926.

Tuberculous alterations were found in the lymph nodes at the angulus venosus in 90 of 100 cases of tuberculosis of the lung. The primary complex of the lung was healed. The fresh tuberculous foci in the lungs of many of the patients were regarded as partly due to an endogenous reinfection caused by a perforation of active tuberculous processes of the angulus lymph nodes into the vascular system of the superior vena cava.

Wilhelm Hueper.

Bronchial Actinomycosis. M. A. Skworzoff, Virchows Arch. f. path. Anat. **261**:503, 1926.

Actinomycotic invasion of the lung usually leads to a diffuse granulomatous process which spreads throughout the lung and may finally reach the pleura or wall of the chest. A much rarer form of involvement is that in which the process spreads along the bronchial tree, causing a catarrhal and purulent bronchitis without marked invasion of the parenchyma of the lung. The author reports an example of this unusual form in a girl, aged 2. The superficial bronchial localization of the process is held to be due to weak pathogenicity of the organism, and the infrequency of the process to the fact that such a weakly pathogenic strain can gain a foothold only in tissues whose resistance has been weakened by other changes.

O. T. Schultz.

SCARLATINA VIRUS. A. SMIRNOWA-ZAMKOWA, Virchows Arch. f. path. Anat. **261**:821, 1926.

In the tissues and bile of persons dead of scarlet fever, the author had seen minute oxyphil bodies which could be stained with strong solutions of eosin. In the belief that these bodies might be the actual virus that prepares the soil for the streptococcus universally present in the disease, attempts at cultivation were made. Bile from the gallbladder was cultured in sugar broth which was kept in the incubator for eleven days without transfer, for the purpose of killing the streptococci present. The broth culture, which contained dead streptococci and their toxin, was injected intravenously into rabbits, from which blood was taken at intervals of six, twelve, twenty-four and forty-eight hours, and injected serially into other rabbits. In rabbits of the third passage, the blood contained numerous minute, highly refractile bodies, some of which were free, but most of which were attached to the surface of red corpuscles. These bodies could be stained with eosin. Guinea-pigs inoculated with second passage rabbit's blood contained similar bodies. With eosin, followed by Giemsa's stain, a few larger basophilic bodies, each with a single central or two poler oxyphil bodies, were also seen. When blood containing the bodies was inoculated into broth, multiplication occurred in the first four or five transfers and then ceased. The author believes that the bodies described by him may be the actual virus of scarlatina.

O. T. SCHULTZ.

GALLBLADDER IN SCARLATINA. A. SMIRNOWA-ZAMKOWA, Virchows Arch. f. path. Anat. **261**:832, 1926.

In 1902, the author had called attention, as the result of his studies of the pathology of scarlet fever, to the belief that the alimentary tract is the primary seat of the infection. Paying more especial attention to the gallbladder in the present investigation, he found streptococci in the mucosa in all except two of thirteen cases. Definite reactive inflammatory changes were present in the gallbladder wall in only three. The oxyphil bodies described by the author could be seen in the wall. He believes that his present observations confirm his earlier ones of the primary invasion of the alimentary tract, and that streptococci reach the gallbladder from the gastro-intestinal tract by way of the lymphatics.

O. T. SCHULTZ.

THE EFFECT OF "SANOCRYSIN" ON EXPERIMENTAL TUBERCULOSIS. B. LANGE and A. FELDT, Ztschr. f. Hyg. u. Infektionskrankh. **106**:692, 1926.

The bacteriostatic action of "Sanocrysin" in vitro varies within wide limits (1:100,000 to 1:25), according to the strain tested. "Sanocrysin" did not have a beneficial effect on the development of experimental tuberculosis in guinea-pigs and rabbits. Seventeen guinea-pigs and seventy-six rabbits were used in these experiments. The dosage of infection, the dosage of the gold salt and the interval between infection and beginning of treatment varied in different groups of animals.

Similar experiments were made on twenty calves. They were infected with a bovine strain of slightly higher virulence than the one used by Møllgaard. Of six control animals, three survived. One made a complete recovery after an apparently severe infection and showed no macroscopic lesions at autopsy, eleven months after infection. The other two were killed two months after infection and showed only slight miliary pulmonary tuberculosis and evidence ot an old healed spontaneous infection. The remaining three controls died

of an extensive exudative pulmonary tuberculosis from nineteen to thirty-four days after the infection. Of thirteen animals treated with "Sanocrysin," nine died of extensive tuberculosis from eighteen to forty-four days after infection; three were killed two months after infection and showed moderately severe lesions, and one killed after eleven months showed no signs of tuberculosis. All four animals that survived the experiment had received Møllgaard's immune serum besides the "Sanocrysin." Thirty-one sheep were infected. Of eight kept as controls, six died between the twenty-ninth and seventy-fourth days; one killed after fifty days and one after three months had moderately severe pulmonary lesions. Eleven were treated with "Sanocrysin" only; ten of these eleven animals died in from thirty-seven to seventy-three days after infection; one showed extensive pulmonary tuberculosis when killed ten months later. Nine sheep were treated with "Sanocrysin" and immune serum. Six died in from thirty-three to seventy-three days after injection; two had moderately severe lesions, and one had extensive lesions at autopsy seventy days and seven months, respectively, after infection. Three animals were treated with serum only; two with the immune serum, one with normal horse serum. They all survived and were killed three and seven months, respectively, after infection; they had minimal or moderately severe lesions. These experiments, therefore, did not furnish any evidence of a therapeutic action of "Sanocrysin" in experimental tuberculosis. The individual variations in the natural resistance of the animals are great, particularly in infections with bacilli of low virulence, and may lead to the interpretation of a therapeutic success, if the experiments are not amply controlled.

MAX PINNER.

DETERMINATION OF THE NUMBER OF LIVING TUBERCLE BACILLI IN BACILLARY SUSPENSIONS BY THE CULTURE METHOD AS COMPARED WITH THE RESULT OF INFECTIONS. B. LANGE, Ztschr. f. Tuberk. **46**:455, 1926.

To standardize bacillary emulsions for graded infections, and to determine the virulence of organisms, it is not sufficient to count the individual bacilli, since this does not determine the number of living bacilli only. Even and extremely dilute suspensions were seeded on egg mediums and injected subcutaneously and intratracheally into guinea-pigs. The number of colonies that developed tallied closely with the number of bacilli in the suspension, determined by the highest dilution which still infected guinea-pigs regularly. The author concludes, therefore, that culturing suspensions of tubercle bacilli on suitable mediums is a sufficiently correct procedure to determine the actual number of living organisms. If a certain amount of a suspension contains at least one living tubercle bacillus, this amount injected into a guinea-pig will produce an infection with certainty. The number of primary pulmonary foci produced by intratracheal injection approximates the number of bacilli in the suspension used, as determined by the culture method. The actual virulence of tubercle bacilli cannot be accurately determined merely on the basis of the weight of the smallest infective dosage unless the number of living bacilli contained in that minimal amount is known.

MAX PINNER.

BACILLUS BRUNKMAN. RUTH HOLMQUIST, Acta. path. et microbiol. Scandinav. **3**:633, 1926.

This bacillus is found not rarely in patients without any intestinal disturbances. In such cases specific agglutinins may occur in the blood, and it seems likely that the bacillus may sometimes be pathogenic.

ON THE HEMOLYTIC ACTION OF B. PROTEUS. N. NISHINA and Y. IKEGAMI, Tohoku J. Exper. Med. **8**:142, 1926.

Of fourteen X strains of *B. proteus* whose two-day culture broths were tested with 5 per cent rabbit blood at 37 C., five were nonhemolytic, and the others produced hemolysis of varying degrees. Filtrates were weaker than the supernatant fluids from centrifugalized cultures. The hemolytic substance was not adsorbed by kaolin, barium sulphate, charcoal or red cells at 0 C., at which temperature hemolysis did not occur, nor was it soluble in chloroform or ether. Cholesterol, strong salt solution and peptone and even lecithin increased its activity, thus suggesting a comparison with the venom of a cobra. Antiserums from rabbits after injections of hemolytic culture broth were not more inhibiting than normal serums; they acted only in low dilutions, and possessed no specificity.

E. B. PERRY.

Immunology

STUDIES IN URTICARIA. I. WHEAL PRODUCTION THROUGH INTERNAL CHANNELS. A. WALZER and M. WALZER, Am. J. M. Sc. **173**:279, 1927.

Wheals simulating those of constitutional urticaria may be prepared by passively sensitizing the skin of the subject with an injection of serum from a hypersensitive patient and then administering the specifically offending food.

ARTHUR LOCKE.

ASPIRATION TUBERCULOSIS IN RABBITS. AN X-RAY STUDY OF THE DEVELOPMENT OF THE TUBERCULOSIS OF PRIMARY INFECTION AND THAT OF SUPERINFECTION. WILLARD B. SOPER, HOMER L. SAMPSON and CHARLES H. HASKINS, Am. Rev. Tuberc. **15**:88, 1927.

The method of pulmonary infection by the instillation of a suspension of tubercle bacilli into one nostril of an anesthetized rabbit affords a simple method of producing tuberculosis of one lung. The localization of the infection is determined chiefly by gravity, the position of the animal being the important factor. The possibility of infection of the nares must be borne in mind. Roentgenograms afford an excellent means of studying the development and progress of pulmonary tuberculosis in the rabbit, and should be more generally employed. An early pneumonic reaction to aspiration of virulent tubercle bacilli may occur in the lungs of both sensitized and normal rabbits. It is more immediate, more intense and more constant in the sensitized animals. The quantity of bacilli introduced is an important factor in the determination of the degree of early reaction to tubercle bacilli in the sensitized lungs. The initial pneumonic reaction may undergo almost complete resolution in both sensitized and normal rabbits. Resolution is more rapid, more complete and more constant in the sensitized animals. Cavities form in both sensitized and normal rabbits, but more rapidly and more extensively in the normal animals.

H. J. CORPER.

A SENSITIZATION METHOD OF DIFFERENTIATING AVIAN FROM MAMMALIAN TUBERCLE BACILLI. A. B. CRAWFORD, Am. Rev. Tuberc. **15**:111, 1927.

A method is described for the differentiation of avian and mammalian types of tubercle bacilli. It is based on the reactions of guinea-pigs, sensitized by avian and mammalian types of tubercle bacilli, to avian and mammalian

types of tuberculins, respectively. The Calmette BCG tubercle bacillus was shown to be of mammalian type. The Friedmann turtle bacillus gave a reaction characteristic of the piscine acid-fast micro-organisms.

H. J. Corper.

Studies in Tuberculosis: VIII. The Identification of a Skin-Reacting Substance in Tuberculous Sera. Frederick Eberson, Am. Rev. Tuberc. **15**:127, 1927.

A heat-sensitive, skin-reacting substance of the nature of a toxin has been identified in the blood serum of tuberculous patients and guinea-pigs. It is not found in normal human or guinea-pig serum. The substance does not behave like tuberculin or its related elements, which are heat-resistant, and it exists independently of them. It is present in largest amounts in advanced tuberculous infections with profound toxemia, and is apparently destroyed by heating at from 60 to 65 C. for from twenty to forty-five minutes. In normal animals this unheated substance gives a positive skin test, owing to its interaction with normal antibodies for tubercle products. The method devised for identifying this specific skin-reacting substance depends, in principle, on a living "indicator," the guinea-pig, which measures related or identical elements in the serum to be tested. The procedure was made possible by a previous demonstration that normal animals could be sensitized with fractional tuberculins prepared from nonprotein substrates. Skin reactions, obtained according to the technic described in these experiments, are referable to an addition or subtraction of one or more of the interacting substances present in tuberculous serum and in the tissues of the experimental animal. Evidence is adduced to show that the heat-labile substance, probably a toxin, in tuberculous serum, can be used as a measure of the circulating antibodies in both normal and tuberculous persons. A test of this type may serve as an index of the bodily resistance to tuberculous infection in both groups. Certain theories and hypotheses regarding tuberculin and skin sensitiveness in tuberculous and normal persons and in animals find an interpretation based on controllable experimental evidence. An explanation is offered for negative tests in advanced tuberculosis, and, furthermore, positive reactions with tuberculous serums in apparently normal persons are attributed to a toxin. Accordingly, the results with the autoserum test of Lenz and with modified autoserum and the Wildbolz reactions take on a somewhat different aspect.

H. J. Corper.

Skin Reactions to Human Serum. Elizabeth H. Chant and Leslie N. Gay, Bull. Johns Hopkins Hosp. **40**:63, 1927.

While unheated human serum caused no reaction on intracutaneous injections in man, serum heated to 56 C. causes an immediate and definite reaction. Guinea-pig serum does not have such an effect.

Erysipelas: VI. Immunization with Soluble Toxin from Streptococcus Erysipelatis Against Recurrent Attacks of Erysipelas. Konrad E. Birkhaug, J. A. M. A. **88**:885, 1927.

1. *Streptococcus erysipelatis* produces a soluble and thermostable toxin when grown in the Douglas tryptic medium digest for a period of five days; the concentration of toxin varies between 10,000 and 50,000 skin test doses per cubic centimeter.

2. During the acute stages of erysipelas, the patient's skin reacts positively with ten skin test doses of *Streptococcus erysipelatis* toxin until about the tenth day of the disease, when the patient's skin reaction rapidly becomes negative to multiple skin test doses of toxin.

3. Simultaneously with the advent of skin immunity to the erysipelas toxin, the toxin that previously was isolated from the patient's blood serum and urine has completely disappeared and a gradually increasing amount of antitoxin appears in the patient's blood serum, which in proper mixtures completely neutralizes the toxin produced by *Streptococcus erysipelatis*.

4. Active immunity against the erysipelas toxin as conferred on the patient by an attack of erysipelas is found to disappear as soon as six weeks after the onset of the disease in certain persons when such persons' negative skin reactions to ten skin test doses of toxin suddenly become positive. Retesting of the patients' blood serum at this time reveals the absence of antitoxic substance.

5. Patients with a definite history of recurrent attacks of erysipelas at frequent intervals are found to contract the disease almost simultaneously with the reappearance of a positive skin reaction to ten skin test doses of toxin.

6. A course of active immunization against recurrent attacks of erysipelas, by means of biweekly intramuscular injections of 500, 5,000 and 50,000 skin test doses of the extracellular and intracellular toxin produced by *Streptococcus erysipelatis*, appears to produce in the patient's blood serum a rapidly increasing concentration of antitoxin, in certain persons, measuring as high as the capacity of 1 cc. of the patient's blood serum being able to neutralize completely 1,800 skin test doses of toxin.

7. Twenty-four patients with definite histories of frequent recurrent attacks of erysipelas have been actively immunized by means of the toxic filtrate of *Streptococcus erysipelatis*, and persons among these who previously suffered habitual attacks of the disease from every sixth to twelfth week have been free from subsequent recurrent attacks over a period approaching two years.

AUTHOR'S SUMMARY.

A CONTRIBUTION TO HELMINTHIC THERAPY. M. FERNÁN-NÚÑEZ, J. A. M. A. **88**:903, 1927.

By injecting extracts of eggs of *Trichocephalus* in dogs an apparent immunity against trichocephaline reinfestation was established.

THE CURABILITY OF SYPHILIS: AN EXPERIMENTAL CONTRIBUTION. ALAN M. CHESNEY and JAROLD E. KEMP, J. A. M. A. **88**:905, 1927.

The results of intratesticular inoculation of rabbits with the lymph nodes of a patient with early syphilis carried out both before and after treatment are reported. Virulent examples of *Spirochaeta pallida* were recovered, by the method described, from the patient before treatment, whereas after treatment and at the end of a probationary period of one year without any antisyphilitic therapy, inoculation of a second lymph node gave negative results. Similar negative results were obtained in the case of two patients who were treated for syphilis early in the course of their disease, and in whom a comparatively long period (four and three-fourths and four years) elapsed between the end of the treatment and the performance of the test. It is desirable that the method of lymph node transfer be applied to the study, in human beings, of given forms of treatment of syphilis.

AUTHORS' SUMMARY.

LOCAL IMMUNITY IN EXPERIMENTAL ERYSIPELAS. HAROLD L. AMOSS and ELEANOR A. BLISS, J. Exper. Med. **45**:411, 1927.

Following endodermal injection of living streptococci in rabbits, the skin involved in the inflammatory reaction becomes resistant to erysipelas streptococci and to some degree also to streptococci from follicular tonsillitis and to virulent *Staph. aureus*. The local immunity spreads to some extent along the lymph channels and with the development of humoral immunity the skin on the uninjected side becomes resistant also.

PRODUCTION OF WASSERMANN REAGINS BY MEANS OF TRYPANOSOMES. K. LANDSTEINER and J. VAN DER SCHEER, J. Exper. Med. **45**:465, 1927.

Injections of dead trypanosomes in rabbits produced strongly positive Wassermann serum. It is suggested that this action may explain the appearance of Wassermann reagins in infections with spirochetes and trypanosomes.

THE PRECISE MEASUREMENT OF HEMOLYSIN. CALVIN B. COULTER, J. General Physiol. **10**:541, 1927.

The hemolysin content is measured as the ratio of the hemolytic activity of a given solution to that of standard immune serum. By this method exact comparison of results obtained at different times with different specimens of red cells and complements can be made. The error is not more than 2 per cent.

THE PROTEIN ASSOCIATED WITH HEMOLYSIN IN RABBIT SERUM AND PLASMA. CALVIN B. COULTER, J. General Physiol. **10**:545, 1927.

The water-insoluble globulin, with which hemolysin is associated, may be separated from immune serum or plasma by dilution and simple dialysis at optimal p_H. This optimum in plasma is influenced by the presence of the fibrinogen. Fibrinogen does not carry any immune bodies, or only an insignificant amount; when present in immune body solutions in other form than fibrin cell, it depresses the hemolytic activity. . . . The hemolytic activity is a more labile property of the immune protein than the agglutinating activity; hemolysin is destroyed, hemagglutinin shows an apparent increase, as a result of alcohol extraction.

AUTHOR'S SUMMARY.

GENERAL AND LOCAL IMMUNITY TO RICIN. ELIZABETH LEE HAZEN, J. Immunol. **13**:171, 1927.

Rabbits are better protected against ricin after intrarectal immunization than after intravenous or intradermal immunization. Intradermal immunization may protect against rectal intoxication but not against intravenous or intradermal intoxication, owing probably to elimination of the toxin through the intestines. Local skin immunity after injection of ricin toxin-antitoxin is not constant.

ACTION OF ENZYMES ON ANTIBODY OF PNEUMOCOCCUS. LLOYD D. FELTON and GLADYS KAUFFMANN, J. Immunol. **13**:219, 1927.

Protective antibodies in water-insoluble protein from antipneumococcus serum were destroyed by proteolytic enzymes.

CLASSIFICATION OF B. BOTULINUS BY PRECIPITIN REACTION. J. B. GUNNISON and P. SCHOENHOLZ, J. Immunol. **13**:237, 1927.

The results agree with those obtained by means of agglutination and fixation. The precipitin test is highly specific for the two types of *B. botulinus* and for the subgroups of each type.

IMMUNIZATION WITH BACTERIA DETOXICATED BY GOLD CHLORIDE. M. B. OSMAN, J. Immunol. **13**:243, 1927.

Gold chloride detoxicates bacteria, but animals injected with such detoxicated organisms tolerate large doses of living organisms; the previous injection of detoxicated bacteria, followed by large doses of live organism, may induce abundant agglutinin production.

SPECIFICNESS OF ALCOHOL SOLUBLE SUBSTANCES OF ACID-FAST BACTERIA. J. Immunol. **13**:265, 1927.

Alcohol soluble substances of acid-fast bacilli show qualitative differences. Serum against mammalian tubercle bacilli contains minor antibodies for alcohol extracts from other acid-fast bacilli, but serum against avian tubercle bacilli reacts only with alcohol extracts from the avian serologic group. Serums against acid-fast bacilli from coldblooded animals and against acid-fast saprophytes react only with alcohol extracts "of those organisms with which a serologic relationship is demonstrable with bacillary suspensions as antigens."

BLOOD GROUPS IN GENERAL PARALYSIS. HENRY A. BUNKER, JR., and SIDNEY MEYERS, J. Lab. & Clin. Med. **12**:415, 1927.

The four groups occurred in ninety-one patients with general paralysis in the same proportions as in the population at large.

A METHOD OF MEASURING THE INCREASE OR DECREASE OF THE POPULATION OF HAEMOLYTIC STREPTOCOCCI IN THE BLOOD. E. W. TODD, Brit. J. Exper. Path. **8**:1, 1927.

In streptococcidal test with defibrinated blood, stationary pipets are unsatisfactory because the cells settle and the cocci multiply in the serum. Continuous movement increases the streptococcidal effect, and there is at the same time a marked increase in the number of cocci taken up by leukocytes.

RÔLE OF RETICULO-ENDOTHELIAL SYSTEM OF THE CUTIS IN EXPERIMENTAL VACCINIA AND OTHER INFECTIONS: EXPERIMENTS WITH INDIA INK. J. C. G. LEDINGHAM, Brit. J. Exper. Path. **8**:12, 1927.

Infiltration of an area of the skin with ink and subsequent vaccination suspends all response by the naked eye. Infiltration with, say, *Strep. erysipelatis* may have the same effect. It is suggested that local defense is increased by stimulation of the reticulo-endothelial cells to proliferate.

AGGLUTININ CONTENT OF TISSUE FLUID. J. S. C. DOUGLAS and F. W. SIMSON, J. Path. & Bact. **29**:441, 1926.

The results of experiments indicate that agglutinin is present in the tissue fluid and can enter the circulation with that fluid, and that it does not do so in a concentration in any way approaching that in the plasma of the blood in response to either of the methods employed—the injection of hypertonic solutions or the use of simple hemorrhage. This is true whether the animals are passively or actively immunized. In animals of the former type there are indications that the concentration of agglutinin in the tissue fluid becomes gradually stronger relatively to that in the plasma, with increasing time after its introduction intravenously. In both actively and passively immunized animals the tissue fluid entering the circulation in response to hemorrhage

has a higher concentration of agglutinin than that which is more rapidly sucked in by the introduction of hypertonic solutions into the blood stream.

The conclusion is therefore that, while the methods employed may have failed to indicate the true concentration of agglutinin in tissue fluid outside the circulation, they have shown that the walls (endothelium) of the blood vessels impose a partial barrier to the passage of this antibody into the blood stream from without.

AUTHORS' SUMMARY.

THE INFLUENCE OF OPTIMAL PROPORTIONS OF ANTIGEN AND ANTIBODY IN THE SERUM PRECIPITATION REACTION. H. R. DEAN and R. A. WEBB, J. Path. & Bact. **29**:473, 1926.

The speed of particulation in a mixture of horse serum and homologous antiserum is dependent on the relative proportions of the two ingredients. In a series of tubes containing a constant volume of antiserum and various volumes of horse serum, the volume of horse serum most favorable to rapid particulation has been determined and differences as small as 0.0001 cc. of horse serum have been detected. By the method of titration which has been described the antibody ratio of any particular antiserum can be expressed as a figure. Thirty specimens of antiserum have been examined. The highest antigen-antibody ratio was 1 to 177, the lowest 1 to 14. The ratio determined with an antiserum and a specimen of normal horse serum has been found to hold good for specimens of horse serum from other normal horses. The method can be employed for the quantitative estimation of either antigen or antibody. The greatest weight of precipitate that can be obtained from any volume of an antiserum is obtained by adding a volume of horse serum greater than that which is found to produce the most rapid formation of particles.

AUTHORS' SUMMARY.

COMPLEMENT FIXATION WITH BILHARZIA. N. HAMILTON FAIRLEY, J. Path. & Bact. **30**:97, 1927.

Injection of alcohol-soluble cercarial extract into goats may give rise to the production of specific complement fixing antibodies. Such extracts, which seem to be free from protein, may provoke a prompt rise in the antibody content of the serum in animals with schistosomiasis.

AGGLUTINABILITY AND AGGLUTINATION. STUDY MADE WITH BRUCELLA MELITENSIS AND BRUCELLA ABORTUS. M. BEGUET, Ann. de l'inst. Pasteur **41**:49, 1927.

Agglutination of bacterial suspensions is due to flocculation of various bacterial substances which are in part adsorbed on the bacterial bodies and in part diffused in the fluid. This flocculation is subject to the general laws governing the stability of colloidal solutions. One substance, which is flocculable in salt solution by heat, acids and nonspecific serums, can be adsorbed by bone black and seems to be a superficial product of the organism. Another substance demonstrable after treatment with bone black is flocculable by bases; and a third substance is flocculable by specific serums, and is a bacterial substance of the genus *Brucella*, which seems to pass in part into the fluid each time that a suspension is made, tending toward an equilibrium of the membrane. The flocculation of this substance is inhibited by a sufficient concentration of the heat-flocculable substance.

G. B. RHODES.

THE WASSERMANN REACTION OF THE MILK OF NURSING WOMEN. H. G. ROTTMANN and H. FRANKEN, Klin. Wchnschr. **6**:66, 1927.

The Wassermann reaction of the milk of lactating women clinically free from syphilis is positive only as long as it contains colostrum.

J. D. WILLEMS.

BLOOD GROUP INVESTIGATIONS. H. SACHS, München. med. Wchnschr. **74**:4, 1927.

This is a lengthy study of blood groups from the standpoints of immunology, biology, blood types, geographic distribution and inheritability.

J. D. WILLEMS.

MECHANISM OF ANAPHYLACTIC SHOCK. G. P. SSACHAROFF, Virchows Arch. f. path. Anat. **261**:751, 1926.

In an investigation of the mechanistic theory of anaphylactic shock of Werigo, who ascribed the shock to multiple pulmonary capillary thrombi which had their origin in intravascular precipitates, the author employed five series of experiments. These consisted of the intravenous injection into rabbits of guinea-pig antirabbit precipitin serum; of the intravenous injection into rabbits consecutively of rabbit antihorse serum and normal horse serum; of the intracardiac, intraparitoneal and intrapleural injection of rabbit antiguinea-pig serum into normal guinea-pigs; of the intravenous injection into rabbits of a precipitate-containing mixture of rabbit antihorse serum and normal horse serum, and of the intravenous injection of a large dose of normal horse serum into rabbits which had been sensitized to horse serum. Only in the guinea-pigs injected with rabbit antiguinea-pig serum and in the final series of sensitized rabbits which received horse serum were any anaphylactic manifestations observed. In these the blood was fluid, the lungs were emphysematous and their blood vessels were engorged, but thrombosis was not present. The experiments did not offer any support for the view that anaphylactic shock is due to pulmonary capillary thrombosis.

O. T. SCHULTZ.

QUANTITATIVE INVESTIGATIONS CONCERNING ISO-AGGLUTININS WITH ESPECIAL ATTENTION TO LEUKEMIA. F. SCHIFF and L. MENDLOWICZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **48**:1, 1926.

The iso-agglutinin titers for various groups were determined and tables as to the frequency for each group are given. In leukemia (thirty-one cases) the iso-agglutinin titer is often surprisingly low.

P. R. CANNON.

INVESTIGATIONS CONCERNING THE CHEMORECEPTOR THEORY: II. THE INHIBITING EFFECT OF PARAFUCHSIN AGAINST TRYPANOCIDAL ARSENICALS. R. SCHNITZER and E. ROSENBERG, Ztschr. f. Immunitätsforsch. u. exper. Therap. **48**:23, 1926.

This work tends further to confirm the "interference phenomenon" of Browning and Gulbransen, and shows the inhibiting effect of parafuchsin against salvarsan and arsazetin when tested with certain strains of trypanosomes.

P. R. CANNON.

CONCERNING ANTIBODIES AGAINST CIRCULATING LIPOIDS AND THEIR SIGNIFICANCE IN THE WASSERMANN REACTION. W. HALBER and L. HIRSZFELD, Ztschr. f. Immunitätsforsch. u. exper. Therap. **48**:34, 1926.

Rabbits were immunized with lipoids extracted from rabbits' erythrocytes, together with swine serum, and produced antilipoids. The serums of such

immunized animals reacted with the lipoids of the "rabbit" and "guinea-pig" type, but did not contain hemolysins for sheeps' erythrocytes. These antibodies are not absorbed by rabbits' erythrocytes. A theoretical discussion of the rôle of antilipoids in the Wassermann reaction is then given.

P. R. CANNON.

CONTRIBUTIONS TO THE KNOWLEDGE OF ISO-AGGLUTININS IN HORSE BLOOD. MAXIMILIAN SCHWARZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **48**:79, 1926.

The iso-agglutination reaction was tested in 100 cases, using horse blood. The macroscopic method was used, 0.95 cc. of citrated plasma with 0.05 cc. of a 25 per cent mixture of washed citrated corpuscles being placed in agglutination tubes. Forty-seven cases belonged to group IV (Jansky) and four to group I. It was not possible to determine the exact groups of the remaining cases.

P. R. CANNON.

THE INFLUENCE OF SPLENECTOMY ON THE THERAPEUTIC EFFECT OF SALVARSAN. N. W. KOLPIKOW, Ztschr. f. Immunitätsforsch. u. exper. Therap. **48**:82, 1926.

In the treatment of mice infected with the organisms of relapsing fever the use of arsphenamine was markedly ineffective in splenectomized animals. In mice infected with *Spirochaeta duttoni,* the percentage of cured animals depended on the length of time before infection that splenectomy had been made. The longer the time before infection the splenectomy had been done, the more nearly did the therapeutic effect of the arsphenamine approach that in the normal mice.

PAUL R. CANNON.

THE RIECKENBERG REACTION IN EXPERIMENTAL RECURRENT FEVER OF THE MOUSE. WALTHER KRANTZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **48**:207, 1926.

Krantz finds that the blood platelets tend to adhere to the spirochetes of relapsing fever in the mouse on the injection of specific immune plasma, but this only applies to living spirochetes. Contrary to the observations of Rieckenberg and others for trypanosomes, the injection of immune serum in the case of recurrent fever gave rise to the blood platelet phenomenon. This phenomenon in vitro does not injure the spirochetes.

PAUL R. CANNON.

THE THERMOSTABLE BACTERICIDAL SUBSTANCES, THE B-LYSINS OF SERUM AND THE BACTERIA INFLUENCED BY THEM. ALFRED PETTERSON, Ztschr. f. Immunitätsforsch. u. exper. Therap. **48**:233, 1926.

Pettersson states that heat resistant bactericidal substances occur in the blood of many animals. These substances consist of two parts, an activable and an activating part, the latter being heat resistant and arising from the thrombocytes and perhaps from other cells. The activable part has a marked affinity for the body cells, so this part can be separated from the activating part by the addition of fresh pieces of tissue to the immune serum. The relationship of these substances to other antibodies is also considered.

PAUL R. CANNON.

ON AUTOHEMAGGLUTINATION. W. D. WYSCHEGORODZEWA, Ztschr. f. klin. Med. **104**:524, 1926.

A man, aged 46, troubled for two years with attacks of weakness, pain in the right hypochondriac region and jaundice, was found to have a greatly enlarged liver, which was smooth, and painful under pressure, a palpable spleen, no fluid in the abdomen and a normal temperature. He denied venereal infection, but used alcohol. His wife was healthy but their eight children had all died within a year of birth. Marked agglutination of the erythrocytes which at first interfered with all attempts at blood examination was avoided by keeping the blood at 37 C., and diluting it only with warm salt solution. The erythrocyte count was low, the fragility tests were irregular and the bilirubin content of the serum was increased. Group II serum (Jansky) agglutinated the red cells rapidly, the serums of groups 1 and III to a lesser degree. Dilutions of the patient's serum agglutinated cells of groups I and III at 1:80, group II cells at 1:40 and the patient's cells at 1:1,600. Autohemolysins were also demonstrated. Serum albumin and globulin were reduced and the protein coefficient disturbed but the relation of this change to auto-agglutination, as claimed by Farhaeus and others, was questioned. The Wassermann reaction was negative; the Sachs-Georgi reaction was ++. The comparative frequency of auto-agglutination with cirrhosis of the liver and with hemolytic jaundice which, according to French authors, is frequently associated with syphilitic infection, and the suggestive family history, prompted treatment with potassium iodide. The erythrocyte count increased to 4,200,000, the fragility became practically normal and the bilirubin content of the serum was decreased.

DISEASES OF THE CENTRAL NERVOUS SYSTEM FOLLOWING VACCINATION. E. FIEDLER, Ztschr. f. Kinderh. **42**:336, 1926.

Three cases of encephalitis following vaccination are reported. Fifty-two instances of disease of the central nervous system after vaccination are said to have been recorded in the literature, and it is suggested that vaccination activates viruses that were present in the system, the powers of resistance being lowered.

A TRANSMISSIBLE AGENT THAT ALTERS THE ISO-AGGLUTINATIVE PROPERTIES OF BLOOD. O. THOMSEN, Hospitalstid. **69**:1154, 1926.

Blood of group I (0) on standing over night acted as of group IV (AB), and a drop of this blood caused this same change in fresh blood. The phenomenon has been maintained for four months by adding a drop of altered blood to suspension of fresh blood, the red cells after thirty-six hours being agglutinated by the serum of any one of the four groups. Each of the four groups acted in the same way. It is assumed that human red cells contain an element that under certain circumstances is made active and reacts with a specific agglutinin present in the serum. This agglutinin is absorbed by the altered red cells. The nature of the transmissible agent that affects this remarkable change in the erythrocytes is unknown. So-called panagglutination and autohemagglutination of which instances are reported, may be examples of the phenomenon described by Thomsen.

THE EFFECT OF THE CULTURE MEDIUM ON AGGLUTINATION. CONRAD BRUNKMAN, Acta. path. et microbiol. Scandinav. **3**:593, 1926.

A new bacillus from the intestinal tract, called *Bac. brunkman*, is described. It is not agglutinated specifically when grown on agar but is highly agglutinable when grown on the Conradi-Drigalski medium. Though not agglutinated

when grown on agar, it nevertheless binds the specific agglutinin. It appears that the development of agglutinability on Conradi-Drigalski medium is due to the lactose present. Several other carbohydrates and alcohols have the same effect as the lactose. Typhoid and the paratyphoid B bacilli and the Shiga-Kruse bacilli may react in some what the same way as *Bac. brunkman*, in that their agglutinability may be raised by the same substances.

A Racial Chart. A Graphic Representation of Iso-Agglutination Results up to the Present Time. Oswald Streng, Acta. Societatis Medicorum Fennicae "Duodecim," vol. 8, 1926.

The author presents a graphic analysis of the percentages of the blood groups found among various races by various investigators. The accompanying illustration is theoretically in three dimensions, although presented as a plane due to the limitations of flat paper printing. Three axes, PP, QQ and RR are used, corresponding to the frequencies of the three inherited factors, p, q and r. The values of p, q and r for each race are given in a table and from these values the position of each race is platted on the graph. The result shows that the various peoples studied fall into natural groups on the graph, the European peoples all being in one part, the Ethiopian peoples in another, the Mongolian peoples in another, the Finno-Ugrians in still another, and so on. The graph is analyzed and discussed in some detail. A considerable amount of original work on blood group distribution among Finns is included in the paper.

Laurence H. Snyder.

Tumors

Ninety Tumors of the Parotid Region, in All of Which the Postoperative History was Traced. Joseph McFarland, Am. J. M. Sc. **172**:804, 1926.

The theory of "enclavement" or accidental sequestration of embryonal cells during the early and complicated development of the face and neck affords the most satisfactory explanation of the origin of their mixed tumors. By that theory it is easy to account for the number and variety of tissues found in the tumors, and for their varying proportions and conditions. Mixed tumors are individual entities, having no relation to the normal structures in which they occur, but from which they do not arise. They have nothing to do with other kinds of tumors, and should be called "mixed tumors" and nothing else, regardless of their histology. They are inherently benign, but commonly recur after excision and if frequently disturbed become locally destructive and invasive, without giving metastasis. The histology of mixed tumors is extremely complex, but on that account their microscopic diagnosis is usually easy. The immaturity, atypical arrangement and confused intermingling of the various tissue components easily lead to misinterpretations as to their nature and mistakes as to their dispositions. Histologic variations among mixed tumors do not have any bearing on prognosis. The microscope, beyond showing that the lesion is a mixed tumor, is misleading rather than enlightening. The rapid enlargement of a mixed tumor of long duration and slow growth is not the result of malignant change. Malignant change, whether "sarcomatous" or "carcinomatous," in mixed tumors must be rare, and its occurrence is difficult to prove. As intervals of ten, twenty and even thirty years may elapse between the operative removal of a mixed tumor and its recurrence; caution should be exercised in declaring any case to be cured.

Author's Summary.

SIMPLE URINE TEST AS AID IN ESTABLISHING MAINTENANCE DIET IN DIABETES MELLITUS. A. GALAMBOS, Ann. Clin. Med. **5**:567, 1926.

Galambos suggests a test of the postcenal glocosuria as a substitute for systematic blood sugar determinations. The postcenal, temporary glycosuria is thought to possess about the same significance as the first elevation of the fasting blood sugar level.

WALTER M. SIMPSON.

LEUKEMIA — A SARCOMA: BONE EVIDENCE. REPORT OF TWO CASES. S. M. CONE, Arch. Surg. **14**:542, 1927.

Many authorities have recognized the similarity between leukemia and tumors, and leukemia has been looked on as a tumor in a fluid medium. Leukemic infiltration of fixed tissue is well known, and its malignant characteristics are recognized. The author feels that the difference between some cases of myeloma with metastases and leukemia is one of consistency rather than a fundamental difference in cell type. To support his opinion, he describes two cases of leukemia in which invasion of brain, liver, intestine, spleen, serous membranes and bone occurred. There was definite invasion of bone by a specific cell of the myelocyte series, which caused bone absorption and also filled the bone marrow.

N. ENZER.

CANCER STATISTICS AS THEY APPEAR TO A PATHOLOGIST. H. G. WELLS, J. A. M. A. **88**:399 and 476, 1927.

Few, if any, facts concerning cancer can be established beyond dispute by the published statistical data. In seeking an answer to such a simple question as, "What is the commonest site of cancer in women?" one will encounter great difficulty, for some large statistical tables put the breast, others the uterus and others the stomach. Clinical observations show that external cancers form the larger proportion of the total incidence of cancer, whereas necropsy statistics indicate that internal cancers are much more common. Too many analyses of medical statistics fail to recognize that postmortem statistics represent a selected group of cases, particularly in respect to cancer.

General vital statistics give a fairly accurate record of external cancers, but an entirely erroneous record of deep-seated cancers. The number of errors in diagnosis, as disclosed by necropsy, is surprisingly high, varying in the reports from different sources from 28 to 50 per cent. Lubarsch believes that about half of the cancer deaths in Germany are not being recorded. A large diagnostic error occurs also in those cases in which the death certificate has been prepared outside of the hospital, particularly as regards the aged, among whom internal cancer is a common cause of death.

Existing statistics are worthless in an attempt to learn anything about the hereditary aspects of cancer. Any attempt to determine the influence of heredity on the occurrence of cancer, utilizing the mendelian principles of inheritance, is destined to failure, because one error in diagnosis could invalidate an entire family record. Further information on the influence of heredity must be obtained by the application of the results of heredity studies, such as those made by Slye on lower mammals.

The proportion of deaths recorded as the result of cancer has been increasing for several years. During this same period, the average span of life has increased, partly from lowered birth rate, and partly from the reduction in the mortality from tuberculosis, typhoid fever, diphtheria and other infectious diseases. In well advanced communities, the high crude cancer death rate gives evidence of the good state of the public health. Every forward step

in diagnosis, hospitalization and medical observation adds to the number of recorded cases of cancer. Another important factor in the increasing cancer rate is the improved recording of vital statistics. Unquestionably, a large number of cases of death from cancer have been recorded as due to "senility and causes unknown."

Wells says that if he were to take of the statistics the only ones that seem to him, as a pathologist, likely to be reasonably accurate, namely, the data on external or accessible cancers, corrected for age and sex, he should have to conclude that in all probability cancer is increasing to just about the extent that people are kept from dying of something else. Little emphasis has been placed on the fact that the increase in the cancer rate is just about the same as the increase in the other three common causes of death in those who have passed the prime of life; namely, cerebral hemorrhage, nephritis and heart disease.

The often repeated statement that cancer is a disease of civilization is not supported by statistics. Relatively few persons reach the cancer age in uncivilized countries. The evidence as to the relative susceptibility to cancer of the peoples of civilized and uncivilized communities, or of the different races, is too fragmentary to permit any positive statements.

WALTER M. SIMPSON.

DISTRIBUTION OF ARSENIC IN TUMOR-BEARING MICE. A. S. MINOT, J. Cancer Research **10**:293, 1926.

Minot describes an accurate method for the determination of small amounts of arsenic. By using this method he was able to study the distribution and excretion of various organic arsenicals after intravenous injection into the tumor bearing mice. He states that, as a group, trivalent arsenicals are retained in the tissue much longer than pentavalent compounds. It is of interest that among compounds of the same valence the presence of halogen atoms in the molecule seems to favor retention. Sulfarsphenamine enters and is retained by the tumors in mice to a greater degree than any other drug studied. The maintenance of a considerable concentration of arsenic in a tumor for several weeks seems not to have any detrimental effect on the cancer.

B. M. FRIED.

THE INFLUENCE OF INSUFFICIENT DIETS UPON TUMOR RECURRENCE AND GROWTH IN RATS AND MICE. KANEMATSU SUGIURA and STANLEY R. BENEDICT, J. Cancer Research **10**:309, 1926.

The rôle of a diet deficient in vitamins, fats, proteins or carbohydrates in the growth of neoplasms has been the object of numerous investigations. Sugiura and Benedict have investigated the rôle of an *insufficient* or restricted diet in the growth of tumors.

Rats fed on a complete basic diet, but limited in quantity to one third of the normal food requirement, survived in their experiments with the transplantation of Flexner-Jobling second carcinoma less frequently, and the tumor grew much more slowly than those of full-fed controls. Under-feeding after engrafted cancerous grafts had well established themselves in hosts did not have a marked inhibitory effect on the subsequent rate of tumor growth. Prolonged postoperative insufficient feeding had a distinct influence on the frequency of recurrences of spontaneous tumor in mice. About 73 per cent of the underfed mice were completely cured of the tumor. On the other hand, only about

18 per cent of the normally fed controls were free from tumor at the time of death. The number of new primary tumors subsequently developed or metastases found at autopsy was less in the underfed animals than on the full fed controls. The interval between the operative removal of spontaneous tumors and development of local recurrences was delayed, and the subsequent rate of tumor growth was diminished in the insufficiently fed animals. The average postoperative longevity in the under fed mice was 142 days against 96 days for the full fed controls. The gain of forty-six days in the experimental animals corresponds to about three and four-fifths years of a man's life.

B. M. FRIED.

NEUROBLASTOMA OF THE ADRENAL GLAND (HUTCHINSON TYPE). REPORT OF A CASE. MAX LEDERER, J. Cancer Research **10**:377, 1926.

Lederer reports a case of a neuroblastoma of the left suprarenal gland found at necropsy in a girl, aged 4½ years. Clinically, the case was characteristic of this variety of tumors in that it showed a left proptosis, radiographic evidences of malignancy in the cranium, and a mass in the left hypochondriac region. From a pathologic standpoint the growth displayed its usual features. It originated in the medullary part of the suprarenal gland. Although nerve fibers could not be demonstrated in the sections, numerous ganglion cells were seen gathered in bunches, or scattered singly among the spindle cells of which the tumor was made up. Curiously enough ganglion cells could not be demonstrated in the metastases only by virtue of the undifferentiated cells. Lederer states that the absence of differentiated cells in the metastases is the chief reason for the occurrence of errors in diagnosis, since the picture usually resembles some form of sarcoma. The condition, in his opinion, is not infrequent, and a more common knowledge of its symptomatology would disclose its presence more often. The roentgenogram of the metastasis resembles closely that of a periosteal sarcoma.

B. M. FRIED.

INFLUENCE OF LIGHT ON THE GROWTH AND MALIGNANCY OF A TRANSPLANTABLE NEOPLASM OF THE RABBIT. LOUISE PEARCE and C. M. VAN ALLEN, J. Exper. Med. **40**:483, 1927.

Under constant light the level of malignancy was low. Under constant darkness the level of malignancy was somewhat lower than in controls under ordinary indoor light conditions, but not as low as among animals constantly illuminated.

OBSERVATIONS ON THE HISTOLOGICAL CHANGES FOUND IN CANCEROUS TISSUES TREATED WITH COLLOIDAL LEAD SUSPENSION. E. E. GLYNN, Brit. M. J. **2**:928, 1926.

As part of a symposium on "The Nature of Malignant Neoplasia and Treatment of the Disease with Lead." Glynn reported on the histologic study of cancerous tissues from patients treated with colloidal lead according to the method of Blair Bell. Only those cases in which material removed by biopsy before treatment was begun could be compared with tissues removed after treatment were used for study. In most of the cases examined histologic changes that might be ascribed to the action of lead could not be detected. Six cases in which changes of one kind or another were seen are described in detail. Glynn's conclusions are as follows: "In the majority of lead-treated

malignant growths so far examined no histological evidence of its action could be detected. In a case of ulcerating cancer of the lactating breast the cancer cells disappeared. In certain other cases of cancer there was histological evidence that lead had increased the regressive changes which usually occur in malignant neoplasms, and in one that it had slowed the rate of growth and so allowed the epithelium to become more differentiated. In another case of cancer of the breast dying from sepsis, remarkable regression of the epithelial cells occurred in metastases examined from the liver, lung and suprarenal; this was less advanced in a metastasis from a fibrosed lymphatic gland, for some of the unaffected epithelial cells even showed mitoses. The changes were chiefly, if not wholly, due to the lead — it is possible that the associated sepsis may have contributed in some obscure way. The lesser degree of regression in the relatively avascular, also fibrosed, lymphatic gland indicates that the action of lead chiefly depends upon a well developed and unobstructed blood supply and perhaps lymph supply. It is histological evidence of an inherent difficulty in treating cancer by lead or by any other drug or by serum. It must be remembered, however, that success or failure of lead treatment may perhaps sometimes depend upon idiosyncrasy of the patient, as in other diseases treated with other drugs."

O. T. SCHULTZ.

FLOCCULATION REACTION FOR SERUM DIAGNOSIS OF MALIGNANT DISEASE. H. J. B. FRY, J. Path. & Bact. **29**:353, 1926.

A saline emulsion of the saline-insoluble acetone-insoluble alcohol-soluble substances of tumor tissue gives a flocculation reaction with the serum of cases of malignant disease. In 1,000 malignant and control cases, 748 correct results were obtained (74.8 per cent). In the 494 cases of malignant disease there were 357 correct results (72.3 per cent), and the 506 controls gave 391 correct results (77.3 per cent). Healthy persons, and pregnant women gave negative reactions, except in two cases each. Nonmalignant tumors gave a negative reaction in 72 per cent of the cases. Syphilis, tuberculosis and certain conditions of cellular disintegration cause flocculation in a percentage of cases. If tuberculosis, syphilis and suppurative conditions are excluded, a positive reaction has considerable weight in the diagnosis of malignant disease. A negative reaction is of less value, but generally indicates that malignant disease, if present, is neither advanced nor widespread. A strongly positive reaction seems to indicate an advanced malignant condition or widespread metastases. The reaction is not completely specific for malignant disease, but is an indication of tissue or cellular disintegration.

AUTHOR'S SUMMARY.

OSTEOCLASTOMA (MYELOID SARCOMA, BENIGN GIANT CELL TUMOR) WITH PULMONARY METASTASIS. E. F. FINCH and H. H. GLEAVE, J. Path. & Bact. **29**:399, 1926.

A case of osteoclastoma of the lower end of the femur, of at least nine years' duration, is reported in which metastases had the histologic characters of the primary tumor, with numerous typical giant cells, and in addition an admixture of fibrosarcomatous tissue. This case, and other cases from the literature, the authors aver, confirm the neoplastic character of these tumors, and leave no doubt that, though usually slow growing and only locally malignant, they are essentially sarcomatous.

AUTHORS' SUMMARY.

THE DEFENSIVE PROCESS OF TISSUES AGAINST CANCER. A. BABES, Bull. de l'Ass'n. française pour l'étude du cancer **15**:381, 1926.

The defensive mechanism of tissues against cancer is unknown. Moreover, it is believed that the organism is entirely powerless against carcinoma, and spontaneous cures from this disease are almost unknown. A. Babes, in his investigation of a large number of carcinomas in Bucharest, noted two methods by which the organism tries to protect itself against cancer.

1. A granulomatous reaction with the formation of pseudotubercles resembling to some extent those seen in tuberculosis, and which he designates as "cancerous pseudotubercles."

2. An "anticancerous phagocytosis" which is evidenced by the appearance of polymorphonuclear neutrophils which invade particularly the cancerous cells.

In support of his thesis, the author gives in detail a histologic description and photomicrographs of tumors that he has studied.

B. M. FRIED.

THE EFFECT OF ARSENIC ON TRANSPLANTED EMBRYONAL TISSUE. M. ASKANAZY, Verhandl. d. deutsch. path. Gesellsch. **21**:182, 1926.

The effect of arsenic on the growth of fetal rat tissue implanted into the subserosa of the stomach of rats was studied. Of eighteen rats in which fetal stomach and intestinal tissue was implanted sixteen showed after four months flat fibrous nodules without any traces of the transplanted parenchyma. In one case a cyst lined by cylindric cells was observed, while in another in the wall of a mucoid cyst a white circumscribed spindle cell sarcoma was present. In a second series of nine rats in which the same operation was performed, but which either received injections of weak arsenic solutions or were fed with Fowler's solution, three animals showed a large adenocystoma, which had lasted more than one year. Atypical squamous cell proliferations were present in one of these cysts. A spindle cell sarcoma originating from the implanted tissue was observed in a fourth rat. Another rat had a large ostoid sarcoma of the hind leg with metastases on the neck. The relation of this tumor to the arsenic feeding remained doubtful. Four factors were considered as necessary for the growth of a tumor: general disposition; local disposition in form of a proliferating tissue due to chronic inflammation, regeneration, hyperplasia and metaplasia; a weak continuous irritation of oligodynamic effect remaining below the threshold of an inflammatory irritation (arsenic); another secondary irritation, being the most important factor, producing the protozoon-like immortality of the blastoma cells. The examination of one of the sarcomas and the metastases revealed a large amount of arsenic six months after the last dose.

W. HUEPER.

TRAUMA AND GLIOMA. BENEKE, Verhandl. d. deutsch. path. Gesellsch. **21**:441, 1926.

Several cases of glioma and gliosarcoma following a mechanical or psychic trauma of the brain are reported. The tumors originate either from the sclerotic glia of the wall of traumatic cysts or from glia cells stimulated to proliferate by chemical changes in the brain caused by the trauma. Tumors occurring soon after the trauma are usually very cellular, while those appearing several years later are hard gliomas. There is postulated a causative relation between trauma and glioma.

WILHELM HUEPER.

CYTOLOGY OF CHICKEN SARCOMA. G. ROSKIN, Virchows Arch. f. path. Anat. **261**:919, 1926.

The Rous chicken sarcoma is composed of large, rounded cells (macrophages, histiocytes), a supporting framework of elongated spindle cells and a variable number of giant cells. Roskin reports a cytologic study of these three types of cells. The normal macrophage has a cytoplasm of uniform density. The rounded cells of the sarcoma (malignant macrophages) have an inner mass of denser cytoplasm and an outer zone of more transparent, probably strongly hydrated protoplasm which forms pseudopodia. The author supposes that the factor which transforms the normal into the malignant cell disturbs the plasmatic equilibrium of the cell, which process makes itself evident in the form of a distinct outer pseudopodia-forming zone, to which the activity of the sarcoma cell is due. The elongated cells which form the stroma are not normal connective tissue cells but fibroblasts which show evidence of irritation. Transitions from the spindle stroma cells to round sarcoma cells were seen. In the giant cells, the cytoplasm had lost its two zones, was homogeneous and contained many fat droplets. The absence of changes in the mitochondrial system of the three types of cells is emphasized.

O. T. SCHULTZ.

REGENERATIVE CHANGES IN THE LUNG IN RELATION TO CARCINOMA. M. BRANDT, Virchows Arch. f. path. Anat. **262**:211, 1926.

Brandt made a study of regenerative phenomena in the lung, with the aim of detecting a possible relation to the pathogenesis of carcinoma of the lung. He accepts Bender's view that in the embryonic lung the cylindric bronchial epithelium contains basal cells and that the alveoli are formed by the active proliferation of the cuboidal epithelium of the terminal bronchiolar bud or pneumonomere. In chronically inflamed human lungs he observed glandlike outgrowths of the terminal bronchioles and proliferation of the basal cells of the epithelium of the larger and medium bronchi. In the lungs of mice exposed to the inhalation of certain irritants he saw similar regenerative phenomena, the proliferation of the bronchial basal cells being the more marked. He subscribes to the Krompecher doctrine of the new formation of cells only from basal or indifferent mother cells. In the Riga city hospital, from 1901 to 1925 inclusive, there were 13,179 necropsies, of which 1,828 were in cases of carcinoma. In the latter group there were 108 carcinomas of the lung. Material from forty-three of these was available for microscopic study. Brandt divides these into three groups, in each of which the tumors had their origin from basal cells of the bronchial epithelium. The first group was composed of tumors in which the basal cells retained their morphologic characteristics and formed strands of narrow, elongated cells. The second group was made up of transitional or mixed cell carcinomas, in which the basal cells by partial differentiation had formed cylindric or squamous cells. In the third group the cells were small and sarcoma-like, but had a tendency to become elongated and take on a palisade arrangement at the periphery of the alveoli or cell nests. The tumors of this group are also held to be of basal cell origin, growth being so rapid that morphologic characteristics of the basal cell are lost.

O. T. SCHULTZ.

MULTIPLE PRIMARY MALIGNANT TUMORS. D. M. CHAJUTIN, Virchows Arch. f. path. Anat. **261**:315, 1926.

The author reports an example of sarcoma and carcinoma in a man, aged 60. The sarcoma, pleomorphic in character, was primary in the upper lobe of the

right lung and metastasized to the wall of the stomach, where an ulcer was present over the metastatic tumor nodule. The carcinoma, of characteristic alveolar structure, was held to be primary in the liver, and metastasized to the lungs, thyroid, pleura, pericardium and axillary, peribronchial and peritracheal lymph nodes.

O. T. SCHULTZ.

PRIMARY CARCINOMA OF THE LIVER. H. ROSENBUSCH, Virchows Arch. f. path. Anat. **261**:326, 1926.

On the basis of a malignant adenoma of the liver of a male child, aged 8½ months, Rosenbusch discusses the origin of primary carcinoma of the liver. The tumor described was limited to the liver, but its malignant character was held to be established by invasion of the blood vessels of the liver. The lobulation of the liver was abnormal, which condition was considered evidence of developmental anomaly. Differentiation into solid cellular strands occurred, but did not result in the formation of typical liver cords. The stroma varied in density and ripeness, its proliferation having occurred not independently but as the result of the primary proliferation of the indifferent liver epithelium. Tabulation of thirty-two previously reported carcinomas of the liver in childhood leads the author to conclude that there is a gradual transition from mixed tumors containing several varieties of tissue plus carcinoma, to tumors composed of epithelium alone, and that all have their origin in abnormalities of development. He postulates further that primary carcinoma of the liver in the adult also arises, not from previously differentiated cells which have undergone anaplasia, but from cells which have remained incompletely differentiated.

O. T. SCHULTZ.

CARCINOMA STATISTICS OF THE AUGUSTA HOSPITAL IN COLOGNE. W. EICHENGRÜN and A. ESSER, Ztschr. f. Krebsforsch. **24**:63, 1926.

The necropsy records of the Augusta Hospital in Cologne for the years from 1902 to 1926 were analyzed by the authors from the standpoint of the occurrence of carcinoma, especially with the view of detecting any effect which the World War may have had. The Pathological Institute of the hospital during the period selected for study was under the successive direction of Borst, Jores, Loeschke and Frank. In 163,525 hospital admissions, there were 13,318 necropsies (8.1 per cent), 932 (7 per cent) of which were in cases of carcinoma. The tabulation of these is of some interest in that it shows an unusually large number of carcinomas of the lung. The more frequent forms were as follows: stomach 255, esophagus 165, biliary tract 114, uterus 69, lungs 68, rectum 50, colon 46, mammary gland 31, ovary 26, prostate 20, and pancreas 20. Any definite influence of the war could not be detected. In the war and postwar periods there was a slight increase in the percentage of deaths due to carcinoma, with an average earlier age at the time of death, whereas the average age at the time of death from all causes was somewhat increased. The percentage of carcinomas with metastasis was also slightly increased, the increase being less in those forms of carcinoma that showed the greatest absolute increase, namely, carcinoma of the colon, rectum, uterus, ovary and female lung.

O. T. SCHULTZ.

REGENERATION AND EXPERIMENTAL TAR CANCER. H. T. DEELMAN and J. P. VAN ERP, Ztschr. f. Krebsforsch. **24**:86, 1926.

In the experimental tar cancer of mice, the tumor arises at one or more small points in a relatively large area which has been treated with tar. Only a comparatively small number of epithelial cells in the treated area developed the characteristics that make them tumor cells. To investigate this peculiarity, the authors made incisions into the treated skin of mice in which the painting with tar had been continued until papillomatous outgrowths had made their appearance or might be expected to appear. Further tar applications were not made. It was found that tumors arose much more frequently in the course of the healing incisions than elsewhere in the treated skin, and that such tumors were more invasive and less papillomatous at an earlier stage than those that developed elsewhere. But even in the regenerating epithelium the change which resulted in tumor formation appeared to be limited to individual cells or groups of cells, because not the entire line of incision was involved in the neoplastic process. Microscopic examination did not reveal any differences between the regenerating cells in the incisions and those in the rest of the treated skin. when the regenerating epithelium in areas 0.5 cm. wide that had been excized from skin areas treated with tar were compared with similar areas in the skin of untreated mice, it was found that the proportion of mitoses situated above the basal layer was larger in the former than in the latter. The authors believe that morphologic examination alone will not permit the detection of the dividing lines between regenerating precancerous and cancerous cells, but that variations of experimental procedures may uncover morphologic changes which at present are unrecognizable.

O. T. SCHULTZ.

ETIOLOGY OF NEOPLASIA. R. BEUTNER, Ztschr. f. Krebsforsch. **24**:99, 1926.

In a paper from the School of Medicine of the University of Louisville, Beutner discusses a number of theoretical factors which may be active in the establishment of neoplasia. All theories of cancer origin resting on a morphologic basis are incomplete and there is no cancer cell as such. The proliferation of cells which manifests itself in tumor formation is the result of chemical factors in the surrounding medium. These probably result first of all in cytolytic changes, to which the multiplication of cells is secondary. The increased potassium and decreased calcium, the lesser electric potential, the formation of lactic acid and the disappearance of salts from their normal localization are looked on as evidences of cytolysis. The causes which bring about alterations in the medium are unknown, but parasites may have such an action. Since the proliferation activating alterations in the medium may be influenced by certain substances which have the property of antagonizing acids and potassium salts, chemotherapeutic control of neoplastic proliferation is theoretically possible.

O. T. SCHULTZ.

SOME PHYSICAL-CHEMICAL CHARACTERISTICS OF CANCER TISSUE. M. A. MAGATH, Ztschr. f. Krebsforsch. **24**:126, 1926.

When transplantable mouse carcinoma tissue was subjected for thirty minutes to the action of isotonic acid buffer solutions of varying hydrogen ion concentration, Magath found that sudden and relatively great swelling of the tissue occurs at a p_H of 4.1. For normal epithelial tissues (liver and renal cortex) the swelling point lies at the same p_H, indicating that there is not any difference between the intracellular p_H of tumor tissue and normal tissue. When

the tissue was treated for thirty minutes with the buffer solutions of varying hydrogen ion concentration and then placed for thirty minutes in distilled water, the minimum swelling occurred in tissues which had been subjected to p_H 5.3 to 4.4. This range was found to be incompatible with the transplantability of the tissue, which appears to indicate the point at which death of the tissue occurs. If the tissues were subjected to 2.5 per cent calcium chloride for thirty minutes before they were placed in acid buffer solutions, the swelling which occurs at p_H 4.1 and below was greatly decreased. In this respect, the tumor tissue reacted like embryonic tissues and differed from normal adult tissues, which were not protected against swelling by calcium chloride.

O. T. SCHULTZ.

COMPARATIVE CANCER MORTALITY IN ENGLAND AND HOLLAND. H. T. DEELMAN and N. M. J. JITTA, Ztschr. f. Krebsforsch. **24**:146, 1926.

In England, the death rate from carcinoma of the mammary gland during the years 1912 to 1920 was 20.4 per hundred thousand of the population, as compared with 10.1 for the Netherlands, and from carcinoma of the uterus, 24.6 as compared with 12.1. The authors analyzed the cancer mortality statistics of the two countries in an attempt to find a reason for the marked differences. The factors considered were operability and extent of the tumor at the time the patient first came under medical observation, social state, number of children, number of miscarriages, heredity, age at menopause, mastitis, trauma and duration of the nursing period. These factors did not appear to have any influence in the varying cancer mortality of the two countries.

O. T. SCHULTZ.

MULTIPLE TUMORS OF DOMESTIC ANIMALS. P. COHRS, Ztschr. f. Krebsforsch. **24**:156, 1926.

Cohr's contribution is of interest to the student of comparative tumor pathology. In 737 necropsies on dogs, he found 70 with tumors, 26 of the tumor group having multiple neoplasms. To these he adds a number of tumors in other domestic animals. His material is subdivided into several groups: multiple tumors of the same kind in the same organ, multiple tumors of the same kind in different organs, multiple tumors of different kinds in different organs, multiple tumors of different kinds in the same organ and combined sarcomatous-carcinomatous tumors. Under each variety of neoplasm described, he gives first the reported examples of the same kind of multiple tumors in domestic animals, with the statement that he does not make any claim for completeness, and follows this with a brief description of his own material relating to the subject. Multiple tumors were more frequent in older animals. Species and race did not appear to be a factor. Multiple maldevelopments, multiple embryonic or postembryonic misplacements and periodic variations in growth energy observed in the tissues of older animals were believed to influence the occurrence of multiple tumors.

O. T. SCHULTZ.

LOCAL EOSINOPHILIA IN CARCINOMA OF CERVIX. E. O. SCHOCH, Zentralbl. f. Gynäk. **50**:2895, 1926.

From the study of 417 patients with cervical carcinoma subjected to radiotherapy, it appears that recovery occurrred far more frequently in the cases with local eosinophilia before beginning treatment than in the cases without such eosinophilia.

Medicolegal Pathology

OILS AND FATS. THEIR ENTRANCE INTO AND FATE IN THE LUNGS OF INFANTS AND CHILDREN: A CLINICAL AND PATHOLOGIC REPORT. HENRY PINKERTON, Am. J. Dis. Child. **33**:259, 1927.

Six instances of oil aspiration in infants are reported. Oily and fatty substances put in the mouth and nasopharynx of infants not infrequently reach the lungs. Here the droplets in the alveoli are taken up by large mononuclear phagocytes and transported by way of lymphatics to the tracheobronchial lymph nodes, while a small amount may pass into the blood. The removal of oil or fat from the alveoli is a slow process in the course of which giant cells and fibrous tissue may form. The most frequent cause of death after aspiration of oily material is a secondary pneumonia. The frequency and importance of this aspiration process here described are not known, but the indications are that it is "not a negligible factor in infant mortality."

CARBON MONOXIDE POISONING: ITS INCREASING MEDICOLEGAL IMPORTANCE. DOUGLAS J. A. KERR, Brit. M. J. **1**:415, 1927.

In Scotland and England as elsewhere the large increase in cases of coal-gas poisoning gives rise to numerous medicolegal questions, one of the most important of which is that of accident, suicide or murder. In this article, which comes from the department of forensic medicine in Edinburgh University, various medicolegal problems associated with carbon monoxide poisoning are discussed, and also the measures that ought to be taken to limit its occurrence. It is pointed out that probably a diminution in the amount of carbon monoxide in lighting gas would result in fewer accidental and suicidal deaths, as there would then be increased likelihood that the victim may be found before it is too late.

MICROSCOPIC CHANGES IN TISSUES AFTER DEATH. K. WALCHER, Centralbl. f. allg. Path. u. path. Anat. **39**:1, 1927.

Sixty-nine bodies, the dates of burial of which were known, were examined. Eight bodies buried in cool weather were free from bacteria at the end of thirty-seven days, and changes in these were attributed only to autolysis. Tissues were fixed in 8 per cent formalin, and sections were studied, unstained and studied in various ways. The conclusions drawn are: the undamaged cell and nuclear membranes, the boundary lamellae of heart muscle fibers, the elastic fibers and lamellae of blood vessels and the elastic fibers of the inguinal canals withstand the ingress of bacteria for a long time; cocci were rarely found in the bodies; early gram-positive rods, spore bearers and pseudoflagellated bacteria were abundant; after months and years spores only were found; in tissues autolysis cannot be sharply differentiated from decay except that in decay gas formation aids in disrupting structural relationships; the oxydase reaction is still positive many months after burial; the fat of fat tissue and the "sudanophile" lipoids are demonstrable a year after burial in an earth grave and eventually the fat tissue changes to fatty acids; intravital fat embolism to the lungs and glomerular tufts can be demonstrated months after burial. Detailed descriptions of decay in collagenic, elastic tissue and cartilage are also given.

G. RUKSTINAT.

THE PRESENT STATUS OF THE LUNG TEST. F. STRASSMANN, Monatschr. f. Geburtsh. u. Gynäk. **75**:47, 1926.

Two cases are described, with photomicrographs, confirming the observations by Fraenckel and Weimann that on the cessation of placental respiration follow-

ing death of the mother, premature respiratory movements on part of the fetus may force amniotic fluid into the fetal lungs. As a result the alveoli may expand so that in the microscopic section the picture resembles that of lungs that have actually breathed air.

SUDDEN DEATH FROM CISTERNA PUNCTURE. F. REUTER, Wien. klin. Wchnschr. **39**:1275, 1926.

The patient, a boy, 14 years of age, with evidences of increased intracranial pressure, died thirty minutes after puncture of the cisterna (magna or cerebellomedularis) under local anesthesia. Fluid was obtained on first puncture, pressure 290 mm. water; as the fluid ceased to flow, repeated punctures were made, but only blood was obtained, the pulse became irregular, and cyanosis developed. At the autopsy a moderate amount of clotted blood was found in the fourth ventricle and also four small hemorrhagic extravasations in the floor of the ventricles; from one of these extravasations a fine puncture canal could be traced into the medulla. The rest of the brain was swollen and edematous; the ventricles were dilated. No tumor or focal disease was found. The symptoms of increased intracranial pressure during life were found to have been caused by a premature synosteosis of the sagittal and lambdoid sutures with chronic hydrocephalus. Undoubtedly the medulla and cerebellum has been crowded down toward the foramen magnum by the pressure.

Technical

THE DETERMINATION OF THE ICTERUS INDEX WITH CAPILLARY BLOOD. D. DAVIS, Am. J. M. Sc. **172**:848, 1926.

The icterus index may be determined by comparing the color of standards with the serum obtained by centrifugalization of a few drops of skin puncture blood in a 2 mm. tube.

ARTHUR LOCKE.

THE KAHN TEST FOR SYPHILIS IN TUBERCULOSIS. GEORGE C. STICKY and W. B. HUNTLEY, Am. Rev. Tuberc. **14**:724, 1926.

An attempt was made to determine whether tuberculosis affects the Kahn test for syphilis. Of 385 patients, 374 who were free from syphilis gave negative reactions. One patient, also believed to be free from syphilis, gave a weakly positive reaction followed by two negative reactions. Another giving a weakly positive reaction could not be checked due to his leaving the sanatorium. The remaining nine patients gave positive reactions. Of this number two gave suggestive and seven definite evidence of syphilitic infection. It appears from these results that the specificity of the Kahn reaction for syphilis is not affected by the presence of coincident tuberculous infection.

H. J. CORPER.

AID IN DIAGNOSIS OF TYPHOID FEVER: A NEW LABORATORY METHOD. C. A. MILLS and K. V. KITZMILLER, Arch. Int. Med. **38**:544, 1926.

Mills and Kitzmiller describe a method of measuring the antithrombin content of the blood and offer it as an aid in the diagnosis of typhoid fever. After blood is drawn from the patient and quickly mixed with a 50 per cent solution of sodium citrate in sufficient amount to make a final concentration of 0.5 per cent it is centrifugalized and 1 cc. of the plasma heated to 60 C. for ten minutes to destroy the prothrombin and fibrinogen. The antithrombin is not affected. Plasma obtained from citrated horse or other blood is now

made to clot by recalcification (0.2 cc. of 1 per cent calcium chloride solution to 1 cc. of plasma), and the serum quickly removed by squeezing the clot in the hand. To each cubic centimeter of the serum is added 0.1 cc. of a 0.1 per cent emulsion of cephalin and one minute allowed for complete activation of the prothrombin to thrombin. The activated serum is pipetted off into tubes (2 cc. to each tube). To the control tube 1 cc. of 0.5 per cent sodium chloride solution is added and to the others 1 cc. each of the heated human plasmas to be tested. At intervals of 2, 5, 10, 20 and 30 minutes 0.3 cc. of each of the foregoing mixtures is added to 0.5 cc. of citrated horse (or other) plasma in a water bath at 40 C. The time required to clot the plasma in each sample represents its thrombic activity, the slower the clotting time the less the thrombic activity. The curve of antithrombic activity reaches its height in from ten to twenty minutes. Taking as the index the ratio of the clotting time of the unknown plasma mixture to that of the control mixture, comparisons of the antithrombic activity of the blood in a variety of conditions were made. Plasma of typhoid patients taken from the beginning of the active period of the disease to complete defervescence of the fever showed a maximum ratio (reached in the 10 and 20 minute readings) of practically 2 or over. This was true in only a few other cases of several hundred samples tested and in these instances the differentiation from typhoid fever was simple on other grounds. The authors consider this phenomenon of heightened antithrombic activity of the plasma of typhoid patients to be constant enough to make their procedure a valuable laboratory aid.

P. S. Rhoads.

The Diazo Test in Nephritis. Harry Blotner and Reginald Fitz, J. A. M. A. **88**:985, 1927.

The test was carried out as described by Hewitt:

To 1 cc. of blood plasma or serum is added 2 cc. of 96 per cent alcohol. The proteins are allowed to precipitate, and the mixture is filtered or centrifugalized. The clear filtrate, or supernatant fluid from the centrifugalized specimen, is then collected. To 1 cc. of this clear fluid is added 0.5 cc. of alcohol and 0.25 cc. of freshly prepared diazo reagent. The mixture is boiled for thirty seconds, and then a few drops of a 10 per cent solution of sodium hydroxide are added.

The characteristic reaction is the very rapid development of a pink color, which may disappear rapidly. The intensity of the color and the rate of its disappearance depend on the concentration of the substance causing the reaction in the blood. The mixture must be watched carefully during the addition of the alkali, since the pink may appear and disappear within a few seconds. The diazo reagent can be prepared according to the directions of McNee. It consists of a mixture of two solutions, each of which keeps well, but which must always be freshly made immediately prior to a test. The two solutions are prepared in the following fashion:

Solution A: sulphanilic acid, 1 cc.; concentrated hydrochloric acid, 15 cc.; distilled water, 1,000 cc.

Solution B: sodium nitrite, 0.5 Gm.; distilled water, 100 cc.

The reagent as used for the test consists of 25 cc. of solution A, to which is added 0.75 cc. of solution B.

The test is positive only in cases of advanced renal insufficiency. It is a test of practical value, being a quick step toward a differential diagnosis between uremic coma and coma of other than renal origin; it is a test of clinical significance as its presence is always an ominous sign, suggesting a short span of life for the patient. In the experience of the authors the test has not been

positive in any nephritic case with a normal phenolsulphonphthalein excretion, though it may develop before there is any significant accumulation of blood nitrogen. On the whole, it appears to be a valuable adjunct to the various renal function tests now generally used, and is particularly to be recommended because of its simplicity and rapidity of performance.

THE CARBON DIOXIDE TENSION OF CEREBROSPINAL FLUID. A. T. SHOHL and S. KARELITZ, J. Biol. Chem. **71**:119, 1926.

A method for determining the carbon dioxide tension of the cerebrospinal fluid is presented, based on the dissociation constant for bicarbonate under the conditions of cerebrospinal fluid. By this method, assuming an uncertainty of 0.02 in the p_H, determination of the carbon dioxide tension may be made with an accuracy of 5 per cent. The value of $pK_1 = 6.13 \pm 0.005$. A compensated acidosis in the spinal fluid may occur simultaneously with a compensated alkalosis in the general circulation. In one case of nondiabetic acidosis a corresponding drop in carbon dioxide tension took place in the serum and spinal fluid. If the same values of p_H and sodium bicarbonate are found for the blood plasma and cerebrospinal fluid respectively, and if the customary values for the dissociation constant and absorption coefficient of blood plasma are used, the calculated carbon dioxide tension of the cerebrospinal fluid is greater than that of the blood plasma by from 5 to 7 mm.

AUTHORS' SUMMARY.

THE PYRIDINE TEST AS A QUANTITATIVE METHOD FOR THE ESTIMATION OF MINUTE AMOUNTS OF CHLOROFORM. W. H. COLE, J. Biol. Chem. **71**:173, 1926.

The pyridine reaction with chloroform and other substances containing the R-C-halogen$_3$ group in the presence of strong sodium hydroxide has been standardized as a quantitative colorimetric method for the estimation of small amounts of chloroform (0.1 down to 0.0001 per cent, corresponding to 0.01256 and 0.00001256 molar), in pure water and in aqueous extracts of animal tissues. Detailed directions for using the test are given, and several checks on the reliability of the method are described.

AUTHOR'S SUMMARY.

METHODS FOR THE DETERMINATION OF SOME OF THE NONPROTEIN SULPHUR COMPOUNDS OF BLOOD. W. DENIS and L. REED, J. Biol. Chem. **71**:191, 1926. NEPHELOMETRIC METHODS FOR THE DETERMINATION OF SOME SULFUR COMPOUNDS IN URINE. W. DENIS and L. REED, ibid. **71**:205, 1926.

Nephelometric micro methods for the quantitative determination of inorganic, total sulphates and total nonprotein sulphur in blood are described, and analyses are presented showing the distribution of these sulphur compounds in human blood and in the blood of several species of animals.

Nephelometric methods are described for the rapid quantitative determination of the sulphur compounds in urine. It is believed that these methods may be of use in cases in which the quantity of urine available is limited to an extremely small volume.

AUTHORS' SUMMARY.

GASOMETRIC MICRO-KJEHLDAHL DETERMINATION OF NITROGEN. D. D. VAN SLYKE, J. Biol. Chem. **71**:235, 1927.

A micro-Kjehldahl method is described in which a quick digestion is obtained by the use of a mixture of sulphuric and phosphoric acids and potas-

sium persulphate, and in which the ammonia formed is determined gasometrically. The neutralized digest is washed into the manometric gas apparatus of Van Slyke and Neill, and is there treated with hypobromite. The nitrogen gas is evolved in two minutes. Results are reproducible to within 1 per cent. The method obviates distillation and the use of standard solutions.

AUTHOR'S SUMMARY.

A NOTE ON THE NATURE OF THE PICRATE OBTAINED FROM NORMAL URINE BY THE METHOD OF FINDLAY AND SHARPE. F. D. WHITE, J. Biol. Chem. **71**:419, 1927.

By the procedure advocated by Findlay and Sharpe for the estimation of guanidine bases in urine, guanidine bases have not been found in four samples of normal human urine. A considerable yield of creatinine potassium picrate has, however, been obtained, and it is regarded as probable that this is the substance obtained by Major and assumed by him to be dimethylguanidine picrate. It follows that no satisfactory chemical evidence exists connecting essential hypertension with retention of guanidine bases. Greenwald's contention that Sharpe's method cannot be used for the estimation of guanidine bases in urine, is upheld.

AUTHOR'S SUMMARY.

THE EFFECT OF POTASSIUM OXALATE ON ELECTROLYTES OF BLOOD AND PLASMA. A. J. EISENMAN, J. Biol. Chem. **71**:587, 1927. METHOD FOR ANAEROBIC DEFIBRINATION OF BLOOD. A. J. EISENMAN, ibid. **71**:607, 1927. A GASOMETRIC METHOD FOR THE DETERMINATION OF p_H IN BLOOD. A. J. EISENMAN, ibid. **71**:611, 1927.

The effect of potassium oxalate on the blood electrolytes is variable and unpredictable. Defibrinated blood, only, should be used for studies involving electrolyte distribution.

A gasometric technic for the determination of p_H in serum or plasma is described, which is claimed to be of wider application and more accurate than the colorimetric methods of Cullen and of Hastings and Sendroy, and less complicated than the colorimetric-gasometric method described by Austin, Stadie and Robinson.

ARTHUR LOCKE.

BACTERIAL FILTERS. S. P. KRAMER, J. Infect. Dis. **40**:343, 1927.

Since all bacterial filters hitherto used have been made of siliceous material of negative electrical charge, it was thought that one might, by using a material of opposite charge, obtain a filter which would remove bacteria, viruses and colloids which have hitherto been classified as filtrable. By the use of calcium carbonate and magnesium oxide of positive electrical charge, this has been accomplished.

AUTHOR'S SUMMARY.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

Regular Meeting, Feb. 10, 1927

ON THE PATHOLOGY OF SULPHUR METABOLISM. E. FOLDES.

My attention was drawn to a possible disturbance of the sulphur metabolism in diabetes by the fact stated by Abel and Geiling that insulin contains sulphur. In order to get additional data, I examined the total sulphur and the ethereal sulphates in the twenty-four hour specimen of urine of patients with diabetes. I found that in the majority of cases there was an increase of the total sulphur and a decrease of the ethereal sulphates, the latter being expressed in percentages of the amount of total sulphur. With this fact confirming the assumption that there is a disturbance of the sulphur metabolism present in diabetes, I was led to the following consideration. Insulin contains sulphur as thyroxin contains iodine, and the effectiveness of insulin depends on its sulphur content as that of thyroxin depends on its iodine content. Furthermore, as biologic reactions can be obtained with iodine similar to those which can be obtained with thyroxin, the possibility of a like effect of insulin and sulphur suggested itself. Sulphur was therefore administered orally to diabetic patients in the form of sulphur precipitatum or ichthyol and the results observed. The oral administration of sulphur reduced the sugar output and the blood sugar of the fasting patient, in the majority of the cases observed, and at the same time reduced the acidosis, if present; in other words, the oral administration of sulphur can increase the tolerance for carbohydrates in patients with diabetes. This effect of sulphur seems to be comparatively more pronounced in those cases in which the sugar output and blood sugar is higher than in cases in which the sugar output and blood sugar is lower. In cases in which there is a low sugar output and a not very high blood sugar, or if sulphur is administered too frequently, the sulphur may have no apparent effect, or may even increase the sugar output and the blood sugar of the fasting patient. Furthermore, sulphur can have a diuretic effect. In three of eleven cases observed, sulphur apparently had no effect on the tolerance for carbohydrates. As signs of hyperthyroidism had been observed in two of these cases, the combined administration of sulphur and thyroid extract was tried, whereupon results were seen similar to those observed in the other cases after the administration of sulphur only. The third patient who did not react to sulphur showed an unusual reaction also to insulin.

The fact that two of the hormones, thyroxin and insulin, contain an inorganic compound similar in their biologic effect to that of the whole hormone, leads to the question whether similar chemical compositions are also to be found in other hormones and whether these inorganic compounds, if present, also act in a manner similar to that of the whole hormone. As it was realized that epinephrine contains an ammonia compound, experiments were made with ammonia on rabbits and it was found that ammonia increased the blood sugar as does the ammonia-containing epinephrine, and also that ammonia produced changes in the differential count of the white blood cells similar to those produced by the injection of epinephrine.

DISCUSSION

MAX GOLDZIEHER: Until now, when sulphur metabolism has been discussed, that sulphur which is excreted as a product of protein metabolism has always been considered, and the idea did not occur that sulphur might also play an active rôle. However, besides the observations of Dr. Foldes, there are other things to be said. The suprarenal cortex contains an unusual amount of sulphur, about 3.6 per cent. The recent work of Loeper in France has shown that the extirpation of the suprarenal increases the sulphur content of the blood, and hence it is concluded that the suprarenal cortex has the capacity of fixing sulphur in the tissues. The suprarenal cortex is the synergist of the pancreas, as it is the antagonist of the suprarenal medulla. This is not generally recognized as yet. There are a number of papers which support this fact, and I believe there is no sound evidence to contradict it. The suprarenal cortex is an organ for the storage and control of sulphur, which suggests a number of correlations that might be of vital importance in the pathology of diabetes.

Another question which Dr. Foldes brought up is the paradoxic action of the simultaneous administration of thyroxin and sulphur. Of course, one would believe that the administration of the antagonist of the pancreas, as thyroxin seems to be, would hinder the effect of sulphur; however, experience with various endocrine substances has shown that such paradoxic observations are not uncommon. I want to call attention to the paradoxic reaction which is seen when pituitary extract and epinephrine are administered together. Either extract given intravenously increases the blood sugar, and if both are given together, the blood sugar does not change. Therefore, two supposed synergists have a different effect than one would expect. The effect is inhibitory. Similar paradoxic observations in endocrinology are common, and do not speak against the value of endocrinology. It shows only that the present knowledge of endocrine pathology is still limited.

Another point of great interest is that endocrine active substances seem to have one radicle which is of inorganic nature. Foldes has pointed out the ammonia radicle in epinephrine. It has been shown also by French workers (Abelous and Soulie) that the epinephrine molecule contains, besides the ammonia radicle, a diphenol and an ethanol radicle. As far as its coloric reactions are concerned, they are due to the diphenol compound, while biologically diphenol is inactive. The amino radicle is active biologically, and does not give color reactions. As to the third radicle of the epinephrine molecule, nothing is known about its biologic or chemical significance. Apparently the complicated structure of the various hormones accounts for the multiplicity of biologic and other reactions connected with them.

LOUIS GROSS: In the first chart, I noticed that Dr. Foldes gives the figures for sulphur excretion in two normal controls, the amount being about 1.1 Gm. a day. Some of the diabetic patients showed about the same amount and there were many more patients examined. I should like to know whether, if Dr. Foldes had examined more normal patients, he might not have found the same variations. An important factor in judging of the effect of sulphur on the metabolism of the carbohydrates would be studies on the respiratory quotient. Has Dr. Foldes made any parallel studies on the respiratory quotient following the administration of sulphur? In observing the diuretic effect which the sulphur seems to have, and in considering the fall in the sugar curve, was this fall in the sugar only a dilution factor, or was it a diminished sugar excretion?

DAVID MARINE: At the present time I cannot agree with Dr. Földes that sulphur occupies as important a position in insulin as iodine does in thyroxin. Abel and Geiling thought that the inactivation and giving off of sulphur after mild alkali treatment predicated that the sulphur group was important. Brand and Sandburg have pointed out that the sulphur in insulin was no more labile than other peptides containing cystine. Insulin contains a high percentage of arginine. Frank and his co-workers have shown that certain guanidine derivatives have a remarkable effect in lowering the blood sugar, and they have now in the market a synthetic preparation of which guanidine is the base, so that the evidence indicates that arginine with its guanidine groups may have more to do with the active chemical group of insulin than sulphur has.

DAVID SEECOF: I thought that when Dr. Marine began to talk concerning the analogy between the iodine in thyroxin and sulphur in insulin, he might mention that when there is an insufficiency of iodine in the thyroid, there is actually no iodine available. Such patients can excrete about a gram of sulphur a day. The patient with so much sulphur in the body should have sulphur available for whatever insulin he needs. In the case of iodine, as I understand it, there is no available iodine. There is an actual deficiency of iodine throughout the body. I do not think a person would have goiter if he could excrete a comparable amount of iodine in the urine. In that way I think the analogy between sulphur in insulin and iodine in thyroxin would not hold.

EUGENE FOLDES: The remarks of Dr. Marine about the rôle of sulphur in insulin are of great interest to me but my experiments are not yet far enough advanced that I can give further information.

Dr. Gross remarked that the sulphur content in the urine was about the same in some of the diabetic patients as in the nondiabetic patients. Of course this is true and I only wanted to point out that in the majority of diabetic patients the amount of total sulphur is higher than in normal patients. There is sufficient data in the literature to show that the normal output of sulphur is between 1 to 3 Gm. daily. Some of the diabetic patients do not show an increase, but the majority do.

I have not as yet made any studies on the respiratory quotient, which would be of great interest.

In regard to the question of the curve of the sugar output meaning only the percentage and being due to a dilution factor, these curves mean the amount of sugar output in grams.

As to the remark that diabetic patients are able to excrete sulphur, as I just pointed out, not only do they excrete sulphur, but they also excrete more than the normal person. This is an interesting paradox, but I think the explanation is that the sulphur, which is given in the form of sulphur precipitatum or of ichthyol, seems to be another form and biologically more active than that which is formed during metabolism.

I was much impressed by the remarks of Dr. Goldzieher explaining the seeming paradox that thyroid extract and sulphur work together, there being a greater influence on the sugar output of the urine and the blood sugar if given together. I think the explanation given by Dr. Goldzieher is the most probable one.

HEMORRHAGES WITHIN THE ISLANDS OF LANGERHANS OF THE PANCREAS. SADAO OTANI. (From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital.)

Observations of hemorrhages within the islands of Langerhans reported in the literature indicate that such lesions occur with special frequency in diabetes, according to some authors, while other authors deny this relationship. The fact that the nature of these hemorrhages has not been investigated by any of the previous workers led to the study of this subject.

The hemorrhages which are studied in this paper are limited to the islands; the acinar structures are not affected. The hemorrhages vary in extent in different islands. If severe extravasation occurs, the insular cell columns are compressed and narrowed by the blood, and partial disappearance of the insular cells may follow.

It is a characteristic feature that the hemorrhages arise only in islands of the pancreas which do not show any other special changes or marked dilatation of the blood vessels in the surrounding structures. It is also characteristic that they usually occur in groups of adjoining islands. However, sometimes only a single hemorrhagic island has been observed in one cross-section of the pancreas. In such instances they may also be found in sections cut from other portions of the same organ. In one severe case there were twelve hemorrhagic islands in one cross-section of the pancreas.

In a series of eighty-five unselected nondiabetic patients, postmortem study of the pancreas disclosed hemorrhagic islands in thirty-six and in thirty of these there was definite evidence of serious infection elsewhere in the body. In six patients capillary hemorrhages were found in other organs, such as the endocardium and lungs. In two patients multiple hemorrhagic infarcts in the lungs were observed.

It may be concluded, therefore, that insular hemorrhages are toxic phenomena due to infection. Similar hemorrhages were found also in the pancreases of diabetic patients; however, most of these patients had died of secondary or terminal infection.

If insular hemorrhages have a definite relation to infectious processes, it is logical to assume that they may arise during life in the course of severe infectious diseases, such as pneumonia, since the hemorrhages appear to be of rather frequent occurrence, and they may finally cause secondary pathologic changes in the insular tissue, namely, atrophy, thickening of the insular capillary walls or fibrosis.

DISCUSSION

DAVID MARINE: Were all of these extravasations of recent origin?

S. OTANI: I could not find any iron pigment.

THE BLOOD PICTURE FOLLOWING SPLENECTOMY IN CASES OF PURPURA HEMORRHAGICA. NATHAN ROSENTHAL.

The blood picture following splenectomy was studied in twenty-five cases, which can be classified as follows: (1) chronic thrombocytopenic purpura hemorrhagica, nineteen cases; eighteen patients recovered and one died; (2) chronic aplastic anemia, three cases; one patient has unimproved and two died; (3) Gaucher's disease, two cases; one patient has improved and one died; (4) leukopenic myeloblastic leukemia and hemolytic icterus, one case; the patient died; (5) tuberculosis of the spleen, one case; the patient died.

The blood picture studied in all these cases consisted of a complete blood count (hemoglobin, red cells, white cells and differential count of the white blood cells), the blood platelets, coagulation time of the blood, bleeding time, capillary resistance (or tourniquet) test and clot retraction. All of the cases showed a thrombocytopenia (from 2,000 to 100,000 blood platelets).

Chronic Thrombocytopenic Purpura Hemorrhagica.—Some cases have been followed as long as three years. The hemoglobin, red cells, and white cells returned to normal in practically all cases; a slight secondary anemia is still present about one year after operation in two cases. In a certain number of patients, especially those that did not receive transfusions prior to the splenectomy, there was a diminution in the hemoglobin and red cells before the rise to normal began. In others, there was an immediate rise of the hemoglobin and red cells. There was a marked leukocytosis in practically all cases, (from 20,000 to 140,000 white cells).

According to the blood platelet reaction, there appear to be two main groups of cases.

(*a*) A thrombocytopenic group in which after a preliminary rise from 20,000 to 300,000 in number, the blood platelets have subsequently returned to their previous low level of less than 100,000. This occurred in ten cases. In three of the cases the bleeding time, tourniquet test and clot retraction gradually returned to normal. In one case the clot retraction is still absent. These patients may be considered as symptomatically cured.

(*b*) In the second group the blood platelets, after a rise varying even up to 1,800,000 in one instance, gradually returned to normal or somewhat above normal. In all these cases the bleeding time, tourniquet test and clot retraction promptly returned to normal status. All these patients have shown a complete cure of their disease.

In the first group the investigator assumes that the disease is primary in the bone marrow with a secondary aggravating factor, such as the spleen. The second group is most likely primary in the spleen which assumes an abnormal function of blood platelet destruction (thrombolytic purpura of Kaznelson).

Chronic Aplastic Anemia.—There was practically no change in the blood picture following splenectomy in the three patients studied, two of whom died within a month after operation. The bleeding tendency has partially subsided in the patient still alive, but the anemia, leukopenia, thrombocytopenia and lymphocytosis are still present.

Gaucher's Disease.—In both cases the blood platelets returned to normal after a preliminary slight rise. The special tests, such as the bleeding time, tourniquet test and clot retraction became normal. The hemorrhagic tendency has become considerably less in the patient still alive.

Leukopenic Myeloblastic Leukemia and Hemolytic Icterus.—There was a slight rise in the blood platelets to 200,000 at first, but otherwise there was no change in the blood picture for the first two weeks. Later the leukocytes increased rapidly to 250,000 with a marked predominance of premature myeloid cells. A typical blood picture of myeloblastic leukemia developed, from which the patient died three months after the operation.

Tuberculosis of the Spleen.—Following the operation, the hemoglobin, red cells and platelets returned to normal. The white cells, however, showed a persistent increase from 60,000 to 100,000 in number for two and one-half years, when the patient died from some cerebral condition. This increase involved the polymorphonuclear neutrophils, lymphocytes and monocytes (leukocythemia). This reaction of the bone marrow is unique and difficult to classify.

DISCUSSION

PAUL KLEMPERER: In connection with the classification of Dr. Rosenthal of purpura hemorrhagica, I want to mention two anatomic observations of mine. In one case of thrombopenia, with a rather acute course in which the autopsy showed no enlargement of the spleen, histologic examination of the bone marrow of the vertebra showed no bone marrow giant cells. In another case in which the patient was operated on and did not die, a great number of platelets were found in the spleen, whereas the first case, which did show an absence of megakaryocytes, did not show any evidence of platelets in the spleen. That, I think, corroborates Dr. Rosenthal's opinion of two types of purpura of the thrombocytopenic type.

STUDIES ON THE DISTRIBUTION AND POSSIBLE FUNCTION OF TISSUE MAST CELLS (A PRELIMINARY REPORT). MAX DEUTCH and DAVID MARINE.

The authors reported on the number and distribution of tissue mast cells in rats following suprarenalectomy. These cells were so named by Ehrich in the belief that they represented connective tissue cells containing stored nutritive material. They are characterized by the presence of numerous closely packed, large cytoplasmic granules which are intensely basophilic and stain metachromatically with several basic aniline dyes, especially after alcohol fixation. The nucleus is relatively small, pale blue staining, and as a rule obscured to a greater or less degree by the cytoplasmic granules. They are thought to be distinct from blood mast cells in mammals, and much has been written as to their structure, origin and distribution in many animal species. Pathologically, little is known beyond the fact that they accumulate around certain chronic local infections and new growths. They are not included in the so-called reticulo-endothelial system, although considered by some as a specialized form of macrophage. As to function, the meager knowledge concerning this might indicate that they may be part of the local defensive mechanism against toxins.

In view of their widespread occurrence in the tissues of the rat and of this animal's strong resistance to toxins before suprarenalectomy and its susceptibility after suprarenalectomy, it occurred to the authors to determine whether there were any changes detectable in the tissue mast cells after suprarenalectomy. The general distribution of these cells was studied in thirty-two rats at varying intervals after suprarenalectomy up to thirty days, and in eight normal rats. The authors report that, contrary to the observations of previous investigators, they found appreciable variations in the number of tissue mast cells in rats of the same age and sex, and that after suprarenalectomy there occurred a diminution in the number of mast cells distributed throughout various tissues. This diminution in number appeared greatest around the fifth day; then there was a gradual rise to normal and even above normal between the fifteenth and thirtieth days. A rapid and simple technic for demonstrating tissue mast cells distinctively for the purpose of rapid general surveys consists of fixation in 80 per cent alcohol, embedding in celloidin and staining with Una's polychrome methylene blue for from ten to fifteen minutes. Undue exposure of the tissues to water tends to dissolve the granules, as has been pointed out frequently.

Since suprarenalectomy in rats causes a marked lowering of resistance to toxins (for example, typhoid vaccine), which occurs about the fifth day, the authors suggest that their observations may indicate some relation between the number of tissue mast cells and the depression of resistance following suprarenalectomy in rats.

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, April 11, 1927

LLOYD ARNOLD, *President, in the Chair*

VARIATIONS IN THE TUBERCLE PARASITE. H. C. SWEANY.

In former reports (*J. A. M. A.* **87**:1206 [Oct. 9] 1926 and *Am. Rev. Tuberc.* **14**:523, 1926), there was uncertainty as regards the interpretation of the observations then recorded. As the result of these and further studies, the monomorphic idea of Koch and his followers was questioned, especially as regards its application to the tubercle bacillus. Having been convinced that the tubercle parasite is not always acid-fast and not always a bacillus, I was led to see if there might not be other changes which either have not been observed commonly or perhaps have not been accepted. The monographs of Löhnis (Washington, D. C., Natl. Acad. Sc., Second Memoir, 1922, vol. 16) and Hadley (*J. Infect. Dis.* **40**:1, 1927), and the various papers by Mellon (*J. Bact.* **11**:203, 1926), are recent summaries of these bacterial changes. The incompletely understood work of D'Herelle (The Bacteriophage and Its Behavior, Baltimore, Williams & Wilkins Company, 1926) may also be mentioned. I shall report only what has been observed with the parasite of tuberculosis.

The nonacid-fast granules of the tubercle bacillus have been changed into acid-fast bacilli which are apparently true tubercle bacilli. The process varies widely, depending, presumably, on the source and environment of the granules. Some reproduce by direct fission, with an elongation of the granules; others lengthen without fission; others unfold giving a "coma" appearance when they first emerge, and some of the granules appear to sprout; that is, the new growth is much smaller than the granule, and it actually takes on a motility that lasts a varying time. Many of the granules contain two, three and as many as five germinating centers. Double granules are common. When these sprout they frequently produce a pair of parallel bacilli.

The acid-fastness of these organisms may return promptly or gradually. I have obtained results with various mediums. Hog spleen tissue incorporated into glycerin agar seems to have a stimulating effect on certain cultures. Petroff's medium without gentian violet is one of the best. When the return of acid-fastness is prompt, frequently an acid-fast coccus or bacillus is joined to the nonacid-fast granule, the newer part taking on acid-fastness. When the return is slower, semi-acid-fast forms of cocci and bacilli with Much granules are seen.

These observations have been accepted rather generally by bacteriologists. Some of the newer aspects of the subject are not so generally accepted, and some have not been presented before, so far as I know. These will be reported as observed, and for the present no conclusions are drawn.

They may be considered as follows:

Degeneration Forms.—These are produced by unfavorable environment, may be semi-acid-fast, may have acid-fast granules or may be wholly nonacid-fast. The size and morphology vary. In fact, it seems that morphology is more a reaction to environment than a specific characteristic. These forms may or may not be tuberculogenic.

Regeneration Form.—These either may be produced artificially, as above, or are found originally in tuberculous lesions. They may be caused to pass back gradually to acid-fastness and, coincidently with this change, the lesions produced in guinea-pigs are like those of tuberculosis.

As further corroberative evidence, I have succeeded in isolating by the single cell method organisms which roughly parallel these in pathogenicity and in staining qualities. A gradual progression of changes seems to take place from the nonacid-fast to the acid-fast, with pathogenic and cultural

properties characteristic of each change. Finally, I have been able to isolate from animals, acid-fast organisms that resemble the human form of tubercle bacillus. It is too early to make this statement final, because the animals are still living. These changes have been brought about more frequently in strains of bacteria that at first were atypical. Nevertheless, the final cultures have been so typical in appearance and so different from the ones at the beginning that I wonder if the start was made with an atypical form, which after several animal passages developed into a typically human type. At present, however, I cannot apply this to tubercle bacilli in general, although one of the strains I employed, which at the beginning was a typically human type, after growth on mannite bouillon produced granular forms in one of the guinea-pigs. These granules assumed various shapes as the environment was altered. I do not wish to generalize on this alone. A large number of organisms must be similarly treated and isolated, preferably by the single cell method, as has been done with the nonacid-fast coccoids and diphtheroid-like forms which have reverted to acid-fastness and have tuberculogenic properties.

A definite rule cannot be given for causing these stable strains to vary. The cultures must be obtained in an unfavorable environment. Then search for unusual forms is made, but no prediction can be ventured when these will appear. Placed in celloidin capsules and buried in the peritoneal cavity of guinea-pigs unusual forms have appeared. These changes have been observed and compared by staining, and by dark-field examination. Being unable to photograph the dark-field preparations I must rely on sketches and written descriptions of the changes.

The apparatus used for single cell purification is the one of Janse and Peterfi made by Carl Zeiss, and a suitable moist chamber which guards against contamination, and enables the accurate isolation of single organisms.

Petroff's egg medium without gentian violet has been used. Gentian violet inhibits tubercle bacilli, especially the regenerating forms. A spleen medium consisting of 5 per cent ground and filtered hog spleen added to glycerin agar, a 5 per cent serum and 5 per cent potato added to glycerin agar, and various other combinations of glycerin, serum and potato with and without agar have been used. Chocolate blood agar is a fair medium for growing tubercle bacilli and also for demonstrating any hemolysis. Various other bouillons and solid mediums have been tried, but plain veal egg mediums rank first. Petroff's medium with gentian violet has little place. Serum mediums, including blood agars of various kinds, are fairly useful; potato medium is less useful. The spleen medium has a tendency to produce anomalous growths but is not recommended for isolating organisms.

The staining methods used have been simple. Good results have been obtained by using simple stains such as Ziehl's, Gram's and a combination of these two in which malachite green is used as a counter stain. All glassware is cleaned in an acid bath, and the stains are filtered before they are used.

DISCUSSION

ERNST PRIBRAM: The tubercle bacillus is a mold related to the actinomyces and accordingly can form a mycelium, bacillary forms or spores. The spores in the tubercle bacillus are difficult to recognize except by staining. Are the red stained portions in the bacilli of the illustration spores or parts of the body? Do these occur in all or just in certain special strains of tubercle bacilli?

H. C. SWEANY: I think the granules are not spores but rather resting stages.

MALIGNANT TUMORS OF THE PITUITARY BODY. E. B. FINK.

Because of the interesting clinical syndromes with which they are associated, tumors were the first lesions of the pituitary body to be intensively studied. Most of these are benign. Of the malignant tumors, sarcomas are extremely

rare. Benda accepts only one report as a true sarcoma. LeCount, in a discussion of acromegaly, emphasizes the absence of metastases as against the sarcoma nature of many of the tumors associated with acromegaly and reported as sarcomas.

True malignant epithelial tumors of the hypophysis are occasionally encountered and are not rare. They are malignant, just as rapidly growing gliomas are malignant. They grow by infiltration of the brain substance, and are often rapidly fatal but do not produce metastases to distant parts of the body.

There are two types of carcinoma of the hypophysis. One is composed of epithelial cells, usually derived from the chief cells of the anterior lobe. The other, the true nature of which was first established by Erdheim, is a mixed tumor derived from the embryologic pathway of the pituitary body, the so-called "hypophysenganggeschwulst." Many of these contain squamous epithelial remnants of Rathke's pouch, some of them have structures resembling thyroid gland tissue, cartilage and bone. Probably all of these malignant tumors arise from the pituitary stalk, and are extrasellar from the outset.

Four tumors are presented in my report. Two were composed of epithelial cells resembling neutrophil chromophobe cells of the anterior lobe, and extended deeply into the brain tissue as far forward as the frontal lobe. The third was a mixed tumor containing bone, cartilage and epithelial structures resembling thyroid tissue. The fourth was a squamous cell carcinoma. All were suprasellar tumors, probably arising in the pituitary stalk.

THE PATHOGENESIS OF AGRANULOCYTOSIS. HARRY JACKSON.

Since 1922, when Werner Schultz first reported the symptoms designated "agranulocytosis," there have been thirty-four reports with the autopsy results from thirty-two patients. Twenty-six patients were from 38 to 65 years old, four were between 17 and 38 and one was a boy 4¼ years old. Most of them were female, only six being male. There was not any history of previous disease. Most of the women were obese, and a number had gallbladder symptoms. One had an alcoholic cirrhosis of the liver; two had had a previous attack from which they had seemingly recovered. The disease seems not to be contagious. Twenty-nine cases occurred in Germany, one in Scandinavia, one in Vienna and three in the United States.

Among the thirty-four patients, pathogenic bacteria were recovered from the blood during life in nine. The pneumococcus and pyocyaneus each were recovered two times, *Staphylococcus aureus* three times and *Bacillus coli* and *Staphylococcus aureus hemolyticus* each once. *Bacillus pyocyaneus* and *Staphylococcus aureus* from two patients, injected into rabbits, caused death in one or two days. *B. pyocyaneus* from another produced a leukopenia in rabbits. These organisms smeared on the throat of animals did not produce any reaction. In most patients the throat during life contained the usual organisms. The bone marrow after death contained *B. coli, B. Pyocyaneus, Streptococcus hemolyticus* and *Staphylococcus aureus* and *albus* once. Many organisms were found in the necrotic places in the throat, and bacterial emboli were found occasionally in the spleen, liver and kidneys.

The characteristic lesions are the gangrenous ulcers of the mucous membranes without granular leukocytes and their precursors in the blood and tissues of the body. The differential blood count demonstrates practically 100 per cent lymphocytes. Ulcers preceded by pseudomembranous inflammation occur in the throat, especially about the tonsils and spread to Waldeyer's ring, the base of the tongue, the larynx, the trachea, the gums, the palate and throughout the intestinal tract. The regional lymph nodes are swollen. The mucous membranes of the vulva, vagina and rectum also are affected. The bone marrow in the long bones is red and has no granular leukocytes and myelocytes. There are numerous nucleated red blood cells and the megakaryocytes are normal. Microscopically, under the necrotic zone of the ulcers, are a few

lymphocytes and plasma cells, but no leukocytes. The oxydase reaction in the bone marrow, spleen and other tissue is negative.

The most probable theory of origin is that there is some toxic agent which destroys the hematopoietic power of the bone marrow to produce granular leukocytes. That is, the myeloblasts and myelocytes are destroyed in the bone marrow as well as the adult granular leukocytes in the blood stream. With this natural defense of the body gone, ulcerations of the mucous membranes occur as a secondary phenomenon. The causal agent is unknown. Another theory is that some specific invading organism is the causal agent and gains entrance to the mucous membrane of the throat. A third theory maintains that there is a congenital deficiency of the bone marrow to produce granular leukocytes. The occurrence of the disease late in life disproves this.

A woman, aged 65, previously healthy except for bronchial asthma, had been under daily observation in the hospital convalescing from a fracture of the neck of the femur. Her blood count before becoming sick was normal. She died on the seventh day of her illness. The lowest leukocyte count was 300 cells per cubic millimeter, 92 per cent of which were lymphocytes. The autopsy demonstrated ulcerative lesions in the throat. The bone marrow had only nucleated red blood cells, megakaryocytes and a few eosinophilic leukocytes.

CONGENITALLY CYSTIC KIDNEYS. H. W. TRAUB.

Congenitally cystic kidneys in a man, aged 47, of at least twelve years' duration, are reported. The left kidney, which filled the entire right side of the abdomen, was removed with the diagnosis of renal tumor. Its weight after inadvertent bisection and loss of at least a liter of cystic fluid was 3,136 Gm. Many cysts, ranging in size from that of a walnut to that of a hen's egg, were present. The intercystic septums ranged in thickness from that of a bare tissue film to several millimeters. The ratio of cyst tissue to solid intervening tissue was easily 3:1. While no semblance of cortical or medullary marking remained, some sections contained many glomeruli, as well as uriniferous tubules, all with retrogressive changes.

After the left kidney had been removed, the right kidney, which had not been felt, could be palpated, although its mass was much smaller. The phenolsulphonphthalein excretion of the right kidney was 42 per cent.

On the twelfth day after the operation, thrombosis of the right leg occurred. Dry gangrene of the right foot followed, and death occurred sixty-five days after operation, and fifty-three after the onset of the thrombosis. The autopsy (C. W. Fishback) demonstrated a right polycystic kidney, weighing 1,108 Gm. Cystic changes were not found in other viscera. There was an abscess at the upper pole of the right kidney, with fibrous adhesions to the diaphragm; a suppurative pyelonephritis, ureteritis and cystitis, and complete occlusion of the right femoral artery by a thrombus distal to the origin of the profunda femoris. There were various and other changes of no significance in causing death.

Book Reviews

LEHRBUCH DER GERICHTLICHEN MEDIZIN. EDUARD R. v. HOFMANN. Elfte Auflage vollständig umgearbeitet von Dr. Albin Haberda o. ö. Professor der gerichtlichen Medizin in Wien. Mit neuer Bearbeitung des psychiatrischen Teiles von Dr. Julius Wagner-Jauregg, o. ö. Professor der Psychiatrie in Wien. Pp. 1234, with 167 illustrations. Vienna and Berlin: Urban and Schwarzenberg, 1927.

This classical work was first published in 1878, and met with such a favorable reception that seven revisions were made by von Hofmann before he died, twenty years later. Its next rewriting was completed by Kolisko and published as the ninth edition in 1905; it was published without alteration in 1909. The tenth, like this last edition, was the work of Haberda. The first part appeared in 1919; the second portion, with consecutive paging, in 1923. The work originated and has since grown to its present success from the abundant material in Vienna, where von Hofmann with his successors and pupils, Kolisko and Haberda, have had charge of medicolegal investigations. It is also a result of the exceptional advantages they have enjoyed in being able to use the material for study in whatever ways they have regarded as essential for research and development of this field of medicine.

Through the profusion of references to the literature and to personal observations by the writers, this book easily has maintained a position for half a century as one of the most competent and authoritative works on legal medicine. During this time, it undoubtedly has been cited in medical literature more frequently than any other single medicolegal work. The store of "source material" it contains is probably richer than that in any other treatise on the subject. Its most conspicuous single feature in marked contrast to other notable treaties of legal medicine is the emphasis given to, and the thoroughness of the descriptions of, gross and minute structural alterations of the human body which are subjects of medicolegal inquiry. The problems in medicine for which investigation is required to assist legal procedure are considered in all the editions in much the same order as in the original work. Their many amplifications have simply kept place with developments. A general review such as this obviously cannot include details of the new knowledge which has been added in the case of many of the widely divergent subjects of medical jurisprudence. There is, however, nothing startling in the way of elaboration in this last edition. The supplementary information has been applied evenly to the various chapters and subdivisions. The work has preserved its former excellent balance. It is natural that wounds from firearms should be given more space, and the huge increase in occupational injuries is presumably responsible for a fuller consideration of the relation they bear to liability and compensation. The portions devoted to sexual potency, sexual crime, pregnancy, abortion and toxicology are much as they were in the tenth edition. From 1,079 pages in the edition of 1905, the increase in pages is only 155, but the illustrations are more than doubled.

The legal responsibility and duties of physicians in Austria and Germany to serve as experts when requested by the judiciary and to conduct investigations, the results of which are designed to promote justice, are set forth with minute details. The laws of both Austria and Germany governing these matters are included in the sections in which the investigations to which they apply are considered. Regret is expressed that the efforts to have such laws uniform for the two countries—efforts spread over many years—have not yet been successful.

The rather elaborate tables in the earlier editions of the recompense fixed by law, item by item, for the services of physicians in these investigations, are omitted. It is impossible to conceive of advances in medical science which shall not be helpful to forensic medicine, since its field is coextensive with that of medicine in general. We may therefore confidently expect the succeeding editions to continue the great reputation this work has long enjoyed.

BEITRÄGE ZUR KENNTNIS DER GENESE DER OVARIAL-EMBRYOM. EXPERIMENTELLE UNTERSUCHUNGEN ÜBER PARTHENOGENETISCHE OVARIALGRAVIDITÄT BEI AMPHIBIEN. By WILHELM BOSAEUS. Pp. 303 with 62 illustrations on 31 plates. Upsala: Almqvist and Wiksells, 1926.

The results of investigation extending over five years in the pathologic institute at Upsala under Quensel are presented. There is a thorough review of the theories advanced for the genesis of ovarian embryomas from a remote past down to recent times. This is not simply a chronologic processional, but a studious résumé with quotations judiciously selected. Then comes an interesting recital of the efforts made to produce these tumors experimentally. These two chapters are a monograph by themselves. The references to literature in them number 285. Their main theme is the parthenogenetic origin of embryomas, but their store of information will serve well for those who desire a recapitulation of the knowledge of the tumors in question. The succeeding chapters contain a full discussion of the author's experiments, to which eighty-nine pages of protocols and the illustrations are appended. The experiments are on a large scale, and the animals used are frogs and toads (*Rana temporia* and *Bufo vulgaris*). The book concerns a study of transplantation, as well as of parthenogenesis. Into the pleuroperitoneal cavity, the dorsal lymph sac or ovaries of 302 adult animals, 2,730 embryos brought to development outside the body with sperm or pathenogenetically were transplanted after they had grown to blastula, gastrula or later stages. The experiments were varied in many ways by homotransplantation and heterotransplantation, by using adults of both sexes as hosts, and by replantation of embryos into the naturally maternal tissues.

In attempts by others to produce such tumors, embryonic tissue has been broken up in different ways before insertion. Bosaeus was careful to harm the living embryos as little as possible in most of the experiments. In spite of a high mortality, cystic teratoid growths and embryomas were obtained in some animals, and found apparently still growing as late as four hundred and seventy-five days after implantation, with no enveloping zone of inflammation. The wide variety of tissues in the tumors thus produced include mucous membranes like those in the alimentary and large respiratory channels, masses of neurologia containing cavities lined with epithelium, retina and other structures of the eye, skeletal muscle, bone, cartilage, osteoid tissue, and glands resembling the thyroid. No cells were found in any of the tumors resembling ova, spermatocytes or their precursors, nor was there any other evidence of gonads. The absence of gonadal structures is repeatedly emphasized by Bosacus. It may be that the prospective supplements to this study will contain a discussion of the relation this feature of embryomas may bear to spontaneous parthenogenesis, hybridization and sterility of hybrids—subjects at present of considerable interest among botanists and students of mutation.

From the results Bosaeus has reported, it does not seem too much to expect that, with further perfection of methods and an improved comprehension of the factors on which success depends, a time may come when embryonal growths may be secured at will in a great number of animals.

Books Received

Die Biologie der Person. Ein Handbuch der allgemeinen and speziellen Konstitutionslehre unter Mitarbeit zahlreicher Fachmänner herausgegeben. Von Prof. Dr. Th. Brugsch und Prof. Dr. F. H. Lewy. Vol. 2 (Lieferung 6). Als Einzellieferung nicht käuflich (single leaflets cannot be bought). Berlin and Vienna: Urban & Schwarzenberg, 1927.

Handbuch der biologischen Arbeitsmethoden unter Mitarbeit von über 600 bedeutenden Fachmännern herausgegeben. Von Geh.Med.-Rat Prof. Dr. Emil Abderhalden. Abt. VII. Methoden der experimentellen morphologischen Forschung, Teil 2, Heft 4 (Lieferung 230). Price, 8.40 marks, in paper covers. Berlin and Vienna: Urban & Schwarzenberg, 1927.

Proceedings of the International Conference on Health Problems in Tropical America, held at Kingston, Jamaica, B. W. I., July 22 to Aug. 1, 1924, by invitation of the medical department of the United Fruit Company. Cloth. Pp. 1,010. Boston: United Fruit Company, 1924.

Arbeten Från Karolinska Institutets Patologiska Avdelning, 1926, Stockholm, 1927.

The Plague Problem in South Africa: Historical, Bacteriological and Entomological Studies. By J. Alexander Mitchell, J. H. Harvey Pirie and A. Ingram. Pp. 256. Johannesburg: The South African Institute for Medical Research, 1927.

Fortschritte der Naturwissenschaftlichen Forschung. Herausgegeben von Prof. Dr. Emil Abderhalden-Halle A.S. Neue Folge, Heft 1. Beiträge zur Geschichte der Erkennung der Beriberi als Avitaminose von Prof. Dr. G. Grijns Landbouwhoogeschool Wageningen, Holland. Pp. 32. Price, 3 marks. Berlin: Urban & Schwarzenberg, 1927.

The Nature of the Intra-Ocular Fluids: Being the Sir Francis Laking Prize, 1926-1927. From the Biochemical Department, St. George's Hospital, London. By W. Stewart Duke-Elder. British Journal of Ophthalmology (Monograph Supplement III). Paper. Pp. 140, with illustrations. London: George Pulman & Sons, Ltd., 1927.

De l'Existence Meconnue de Diverse Plexus Neuro-musculaire, Segmentes. Distributeurs des Incitations dans l'Intimite due Myocarde. Henri Schlossmacher. Porto Alegre, Brazil: Typographia Santo Antonio, 1926.

Annual Report of the County Physician of the County of Essex, State of New Jersey, for the Year Ending Dec. 31, 1926.

Die Serodiagnose der Tuberkulose. Von Dr. Max Pinner, Direktor des Laboratoriums der Städtischen Tuberkulose — Heilstätte Detroit, Mich. Pp 88. Price, 5 marks. Leipzig: Johann Ambrosius Barth, 1927.